

Fish, Docosahexaenoic Acid and Alzheimer's Disease

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Abbreviations:

A β – amyloid beta

AD – Alzheimer's disease

ApoE – apolipoprotein E

ARA – arachidonic acid (20:4 ω 6)

DHA – docosahexaenoic acid (22:6 ω 3)

EPA – eicosapentaenoic acid (20:5 ω 3)

GLUT1 – glucose transporter 1

MCI – mild cognitive impairment

MMSE – mini mental state examination

NPD1 – neuroprotectin D1

PE - phosphatidylethanolamine

PPAR – peroxisomal proliferator-activated receptor

PUFA – polyunsaturated fatty acid(s)

RAR – retinoic acid receptor

RCT – randomized clinical trial

RXR – retinoid X receptor

TG – triglyceride(s)

Key words: Alzheimer's disease, docosahexaenoic acid, fish, aging, brain, ω 3 fatty acids, eicosapentaenoic acid, cognition, dementia, cognitive decline, arachidonic acid.

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ABSTRACT

Cognitive decline in the elderly, particularly Alzheimer's disease (AD), is a major socio-economic and healthcare concern. We review here the literature on one specific aspect of diet affecting AD, that of the ω 3 fatty acids, particularly the brain's principle ω 3 fatty acid - docosahexaenoic acid (DHA). DHA has deservedly received wide attention as a nutrient supporting both optimal brain development and for cardiovascular health. Our aim here is to critically assess the quality of the present literature as well as the potential of ω 3 fatty acids to treat or delay the onset of AD. We start with a brief description of cognitive decline in the elderly, followed by a brief overview of well recognized biological functions of DHA. We then turn to epidemiological studies, which are largely supportive of protective effects of fish and DHA against risk of AD. However, biological studies, including blood and brain DHA analyses need careful interpretation and further investigation, without which the success of clinical trials with DHA may continue to struggle. We draw attention to some of the methodological issues that need resolution as well as an emerging mechanism that may explain how DHA could be linked to protecting brain function in the elderly.

1. INTRODUCTION

Cognitive decline, particularly in the form of Alzheimer's disease (AD), has emerged in the past 20 years as a major challenge to the quality of life for the elderly and their caregivers. AD and neurodegenerative diseases in general are putting significant pressure on healthcare resources both in developed and developing countries. Once it is clinically diagnosed, there is little prospect of improving the prognosis of AD [1]. Hence, to reduce the risk of cognitive decline in the elderly, closer attention needs to be paid to the possible impact of lifestyle and other non-genetic, hence modifiable, risk factors that are present in younger adults. Diet quality is a lifestyle risk factor contributing to AD and chronic degenerative diseases in general. One class of dietary nutrients closely associated with optimal function of the brain is the ω 3 polyunsaturated fatty acids (PUFA), particularly docosahexaenoic acid (DHA; 22:6 ω 3).

We review here the possible link between fish intake, DHA and declining cognitive function associated with aging, with emphasis on three types of studies in humans – epidemiological studies of fish or DHA intake, measurement of blood or brain DHA levels, and clinical trials of supplements containing DHA in age-related cognitive decline, AD, or other forms of dementia. Animal studies linking DHA to AD will be described briefly but have recently been discussed elsewhere [2, 3] and are therefore not the focus of this review.

2. DECLINING COGNITIVE FUNCTION IN THE ELDERLY

2.1. Clinical Aspects

Dementia is defined as a clinical syndrome which associates a memory disorder and impairment in at least one other cognitive domain, both of which significantly interfere with social function or activities of daily living [4, 5]. AD is the most frequent cause of dementia, accounting for 50-60% of all cases [1]. It is characterized by a progressive decline in memory function with objective evidence of impaired episodic memory on neuropsychological testing.

Aside from the presence of certain predisposing genetic polymorphisms, several clinical features can be used to support the diagnosis of probable AD. These include the presence of atrophy in the medial temporal lobe and/or hippocampus on brain imaging, biomarkers in cerebrospinal fluid (see Section 2.2 - Neuropathological Features), or specific abnormal patterns of brain glucose uptake seen with positron emitting tomography [5]. The diagnosis of AD is considered definite only when specific neuropathological criteria are met (see below) or when genetic evidence (mutation on chromosome 1, 14 or 21) is present in addition to the clinical criteria cited above [5].

Before the dementia stage, AD pathology may manifest through subtle cognitive decline greater than expected for an individual's age and education but with minimal impact on daily living. This transitory and still reversible stage is usually termed mild cognitive impairment (MCI), but there is as yet no clear consensus on its definition [6-9]

2.2 Neuropathological Features

Senile plaques and neurofibrillary tangles in the medial temporal lobe structures and cortical areas are the neuropathological hallmark of AD [1]. AD is characterized by the accumulation of various β -amyloid ($A\beta$) peptides resulting from the cleavage of the amyloid precursor protein, in particular peptides composed of 40 ($A\beta_{40}$) and 42 ($A\beta_{42}$) amino-acids. $A\beta$ is produced constitutively during normal cell metabolism but under normal conditions, brain $A\beta$ does not accumulate. According to the amyloid cascade hypothesis [10], the central event in AD pathogenesis is an imbalance between $A\beta$ production and clearance, with increased $A\beta$ production in familial AD and decreased $A\beta$ clearance in sporadic AD [1].

There are several pools of soluble and insoluble $A\beta$ that are normally in equilibrium. Soluble forms of $A\beta_{40}$ and $A\beta_{42}$ have toxic effects and are thought to be responsible for the

severity of the neurodegenerative process in AD [11]. Conversely, the level of insoluble A β , which is a major part of total amyloid load, distinguishes AD from controls but is not correlated with disease severity or number of amyloid plaques [11]. Soluble A β species constitute a fluctuating intra- and extra-cellular pool whereas insoluble A β is usually viewed as relatively stable.

The other characteristic lesion of AD is the presence of intra-cellular neurofibrillary tangles composed of abnormally hyperphosphorylated tau protein. Tau pathology is generally considered as a downstream event but may contribute to neuronal dysfunction and cognitive impairment [1]. AD pathology is also accompanied by synaptic dysfunction and neuronal apoptosis.

2.3 Risk Factors

Old age is the main risk factor for AD since the prevalence of AD increases almost exponentially from 1% at 60-64 years old to 25 % or more at 85 years old and older [1]. The presence of certain predisposing genetic polymorphisms can also greatly increase the risk of AD. Mutations in the amyloid precursor protein, presenilin 1 and presenilin 2 genes have been identified in the familial form of AD, a rare autosomal dominant disorder with onset before 65 years old. In sporadic late-onset AD, presence of the ϵ 4 allele of the apolipoprotein E gene (ApoE4) is a significant predisposing factor which lowers the age at onset [12] (see also Section 6.2.2 - Apolipoprotein E).

Risk of AD is also associated with reduced brain 'reserve capacity', a parameter linked to lower education and insufficient activities that are cognitively and/socially stimulating, i.e. puzzles, crosswords or card games [1]. Risk factors for vascular disease also increase the risk for AD, including hypertension, dyslipidemia, atherosclerosis, smoking, diabetes, obesity

[1]. Conversely, nutritional factors such as anti-oxidants, B vitamins, and ω 3 PUFA such as DHA may have a potentially protective effect [13-15].

3. BIOLOGICAL FUNCTIONS OF DHA

As the biological functions of DHA have been the matter of a recent extensive review [3], here we will only briefly summarize putative mechanisms of action by which it might reduce the risk of cognitive decline or AD. In addition to specific functions in the brain, DHA has several non-specific properties which could also potentially contribute to its protective effect against the neurodegenerative process in AD.

3.1 Neuronal Membranes

DHA is a major component of the lipids in neuronal membranes where it clearly exerts a structural and functional role. The physical state of the neuronal membrane is critical in the control of transfer of the neuronal information, i.e. neither too rigid nor too fluid for the ionic exchange between the inner and outer walls of the membrane [16]. DHA also modulates properties of the hydrophobic core of the membrane bilayer, including conferring a high degree of flexibility and directly interacting with membrane proteins, thereby impacting on speed of signal transduction, neurotransmission, and formation of lipid rafts [17].

3.2 Amyloid β

Many in vitro and animal studies suggest a direct role of DHA on A β [2] (see also Section 7 - Animal Models) In various animal models and using a variety of experimental protocols, DHA exerts a protective effect against neuropathological signs of AD, including A β accumulation, synaptic marker loss, and hyperphosphorylation of tau [18]. The DHA-derived

mediator, neuroprotectin D1 (NPD1), is formed in the brains of AD patients as well as in human brain cells in culture. In cytokine-stressed human neural cells, DHA attenuates A β secretion, an effect accompanied by the formation of NPD1. NPD1 promotes brain cell survival via the induction of anti-apoptotic and neuroprotective gene-expression programs that suppress A β 42-induced neurotoxicity [19]. Enrichment of membranes with DHA protects neurons in culture against apoptosis when exposed to soluble A β [20]. This protective effect depends on the way the experiment is conducted; pre-treatment with eicosapentaenoic acid (EPA, 20:5 ω 3) and DHA can actually reduce the survival of neurons incubated with A β 42, but this deleterious effect was not observed when EPA and DHA were added at the same time as the A β 42 peptide [21].

In living human beings, A β measurements can be performed in CSF or in plasma, the most convenient fluid to sample for clinical studies. Despite controversy regarding their ability to predict AD, plasma A β 40 and A β 42 concentrations and their ratio have been proposed as a measure of the impact of treatments in clinical AD trials [22, 23]. The possible efficacy of DHA in the treatment of AD could therefore potentially be evaluated by its ability to reduce the plasma level of soluble A β [11]. However, to our knowledge, A β concentration has not yet been measured in any of the few clinical trials assessing the impact of ω 3 PUFA supplementation against cognitive decline or dementia in humans (see Section 5.3 - Clinical Trials).

3.3 Cardiovascular Properties

The protective effect of EPA and DHA in cardiovascular disease is well documented in humans [24], in particular through randomized controlled trials (RCT) [25-28]. Elevated plasma triacylglycerols (TG) are a component of the metabolic syndrome which is linked to higher risk

of cognitive decline, in particular in the presence of elevated inflammation [29]. More specifically, elevated plasma TG are a risk factor for dementia, in particular of the vascular type [30, 31]. In obese mice, lowering TG with gemfibrozil reversed cognitive impairment [32].

One of the main effects of dietary ω 3 PUFA is to reduce elevated plasma TG [28, 32-41]. The hypotriglyceridemic effect of ω 3 PUFA is believed to be due mostly to their potent enhancement of lipolysis through activation of peroxisome proliferator-activated receptors (PPAR; see Section 3.6 - Nuclear Receptors) [38]. Therefore, if supplemental DHA or EPA were to be shown to have a protective effect against cognitive decline or AD, the mechanism might well involve TG lowering.

3.4 Anti-inflammatory Properties

Inflammation plays a major role in the development and progression of AD. The accumulation of A β in AD is accompanied by an inflammatory response resulting from microglia activation and recruitment of astrocytes, inducing the expression of pro-inflammatory cytokines [23, 42]. In turn, cytokines stimulate A β synthesis and amyloid formation, setting off a vicious circle of exacerbation of inflammation [23]. Overproduction of reactive oxygen species by brain cytokines increases oxidative stress and hence the risk of neurodegeneration [43]. Transient acute systemic inflammation also increases the vulnerability of the brain to neurodegenerative disease [44].

Long-chain PUFA are viewed as potent modulators of inflammation. Most of the mediators formed from EPA and DHA (leukotrienes, resolvins, NPD1) are anti-inflammatory, whereas those formed from the ω 6 PUFA, arachidonic acid (ARA, 20:4 ω 6) are mostly pro-inflammatory [3, 45]. The anti-inflammatory effects of ω 3 PUFA could therefore contribute to their protective effect against neurodegeneration.

Very few RCT have assessed the impact of DHA or EPA supplementation on peripheral inflammatory markers in various diseases and the few that have been reported are of small sample size and have shown inconsistent results [36, 46-50]. In the OmegAD trial conducted in patients with mild to moderate AD receiving 1.7 g DHA and 0.6 g EPA daily, increased plasma concentrations of EPA and DHA were associated with reduced release of interleukin-1 β , interleukin-6 and granulocyte colony-stimulating factor from peripheral blood mononuclear cells [51], results supporting a dampening effect of EPA and DHA on peripheral inflammatory markers.

3.5 Oxidative Stress

Oxidative stress results from an imbalance between formation and degradation of pro-oxidants or decreased cellular antioxidant protection mechanisms, and may result in increased cell damage and apoptosis. Excessive oxidative stress stimulates multiple signalling pathways in the central nervous system that then participate in pathophysiological processes leading to cell damage and, eventually, cell death [52]. The brain is particularly susceptible to oxidative stress because, on the one hand, it has a high content in easily peroxidizable long-chain PUFA, in particular DHA and ARA, and on the other hand, because mitochondrial function to fuel the brain's normal energy requirements consumes a lot of glucose, a process associated with relatively high production of free radicals [53].

In AD, the accumulation of A β is associated with increased free radical production and increased lipid peroxidation in the brain [53]. In post-mortem brain samples from AD patients, oxidative damage can be observed in the form of oxidized lipids, proteins and DNA (Choi et al. 2004 J Biol Chem). Important oxidative damage has also been observed in subjects with MCI, suggesting an early role of oxidative stress [54].

DHA administration exerts antioxidant activity as shown by increasing glutathione reductase activity and decreasing accumulation of lipid peroxide and reactive oxygen species in the cortex and hippocampus of AD model rats [55, 56]. Moreover, a transgenic mouse model of AD given an ω 3 PUFA deficient diet exhibited increased oxidative damage [57]. Some animal models of ischemia-reperfusion brain injury show significant free radical scavenging capacity of DHA and protection against peroxidative damage of lipids and proteins in developing and adult brains, with attenuation of neuron loss and cognitive and locomotor deficits [17]. However, in a cellular model, DHA did not prevent the oxidative stress induced *in vitro* in neurons exposed to soluble A β peptides [20, 42].

The few human studies examining the impact of DHA supplementation on oxidative stress have yielded inconsistent results. In a double-blind controlled trial, 59 subjects were randomized to 4 g/d of purified EPA, DHA, or olive oil for 6 weeks while maintaining their usual diet. In one study, urinary F₂-isoprostanes, a measure of lipid peroxidation, were significantly decreased by dietary EPA or DHA in type 2 diabetic subjects [47]. Plasma 8-iso-PGF_{2 α} was significantly decreased after 3 months of supplementation with ω 3 PUFA [58], also suggesting inhibition of ARA metabolism to eicosanoids and/or a possible antioxidant effect of EPA and DHA. However, supplementation of healthy adult men with high dose of EPA and DHA (total 5.3 g/d) may also increase lipid peroxidation, an effect not suppressed by high dose vitamin E (900 IU/d) [59]. Therefore, DHA supplementation at low doses may be protective against oxidative stress, whereas higher DHA doses promote lipid peroxidation [60]. The DHA response to oxidative stress may also be linked to the production of NPD1 generated enzymatically from unesterified DHA. NPD1 may in turn inhibit oxidative stress or have other neuroprotective functions at the site where the free DHA is produced [61, 62]. NPD1 production may be influenced by redox balance and availability of other antioxidants.

3.6 Nuclear Receptors

Changing fatty acid intake, particularly PUFA, is an important means of modulating gene expression in various tissues [63, 64]. EPA and DHA interact with at least four families of transcription factors – peroxisome proliferator-activated receptors (PPAR), liver retinoid X receptors, hepatic nuclear factor-4 α and sterol regulatory-element-binding protein. EPA and DHA also generate a large range of eicosanoids modulating transcription factor activity [65]. PPARs are ligand-inducible transcriptional factors with a central role in regulating gene expression. PPARs belong to the class II superfamily of nuclear receptors which also includes retinoic acid receptors (RAR). RXR, the receptor of 9-*cis* retinoic acid, is considered as the common dimerisation partner of several other nuclear receptors in particular PPARs [66].

Among the three known isoforms of PPAR (α , β/δ and γ), PPAR γ is the most studied, partly because of its ability to inhibit inflammatory gene expression in brain cells [67, 68]. PUFA bind PPARs with a higher affinity than saturated or monounsaturated fatty acids and induce gene transcription by interacting with distinct DNA promoter sequences in the target genes predominantly in the form of PPAR/RXR heterodimers [69, 70]. Modulation of the expression of genes targeted by PPAR may well also be affected by dietary intake of ω 3 PUFA because of the high efficacy of ω 3 PUFA as PPAR ligands [3, 71].

RAR and RXR play key roles in many aspects of brain development, including neurogenesis during embryogenesis, morphological differentiation of catecholaminergic neurons, and activity-dependent plasticity [17]. RAR and RXR are also highly expressed in the hippocampus, which may be relevant to understanding the role of DHA in AD. Several studies show that DHA can specifically bind to and directly activate RXR, leading to the regulation of the expression of genes usually under the control of retinoic acid [72-74]. These results may

have potential cerebral consequences since the retinoic acid signalling pathway is involved in the maintenance of synaptic plasticity and mnemonic performances in aged animals [75-78]. In humans, a link between the metabolism of retinoids and late onset AD was recently proposed [79, 80].

4. EPIDEMIOLOGICAL STUDIES

4.1 Prospective Studies

We turn back now from the biological functions of DHA to its possible role in the link between fish, ω 3 PUFA intake and AD. The strongest support for a causal association between low fish and/or low DHA intake in AD comes from prospective epidemiological studies conducted in France, the Netherlands, Scandinavia, Italy and the USA. Of the nine such prospective studies that have been published, most show that higher intake of fish or long-chain ω 3 PUFA decreases the risk of cognitive decline, dementia or AD in the elderly (Table 1).

The Rotterdam Study was the first to report that fish intake protected against the risk of AD [81]. However, at just 2 years, the initial follow-up period of this study was relatively short. In fact, in the subsequent 6 year follow-up of this cohort, no significant relationship was observed between fish intake and risk of AD or all cause dementia [82]. A lower risk of incident dementia or cognitive decline in those consuming more fish has nevertheless been reported in several other independent prospective cohort studies [83-88].

Only one prospective study has not shown a significant benefit of higher fish intake and risk of AD [89]. This study was based on a subsample of 488 participants representing about 25% of the main cohort in which both dietary DHA intakes were calculated and plasma DHA measurements made. A protective effect of higher plasma DHA was observed against the risk

of all cause dementia but not for AD. This negative finding for AD *per se* may have been due to a lack of statistical power because the 50% risk reduction for AD in subjects consuming fish more than twice a week was almost statistically significant (95% CI 0.20-1.27; $P=0.14$).

Furthermore, several studies suggest that polymorphisms in Apolipoprotein E (ApoE) may inhibit or prevent the beneficial effect of fish in reducing the risk of cognitive decline in the elderly (see Section 6.2.2 - Apolipoprotein E).

4.2 Cross-Sectional Studies

Three cross-sectional studies describe better cognitive performance on various neuropsychological tests in middle-aged [90] and older persons [91, 92] who regularly consumed fish. In these studies, consumption of fish other than fried fish was also associated with lower prevalence of subclinical infarcts and white matter abnormalities on brain images in older persons, thereby indicating a lower risk of vascular dementia [93]. Despite the association between higher fish consumption and better cognitive performance reported in these cross-sectional studies, this type of study cannot ascertain causality, i.e. whether the observed dietary behaviour is the cause or the consequence of cognitive impairment. Indeed, dementia is accompanied by a progressive loss of autonomy in activities of daily living which impairs the ability for shopping, preparing adequate meals, and eventually eating well. Hence, the value of observational epidemiological studies in understanding the link between dietary intake of fish or DHA and risk of dementia or cognitive decline depends crucially on their design.

4.3 Fish Intake Compared to DHA Intake

DHA intake is normally a direct function of fish and seafood intake [89, 94, 95]. The two principal reasons for this direct association are that – (i) on a population basis, there is almost

no other dietary source of DHA other than fish or seafood and, (ii) the conversion rate from α -linolenic acid and EPA to DHA through the desaturation-chain elongation system is very limited in humans and has essentially no impact on plasma DHA [96, 97].

Despite the clear association between fish and seafood intake and DHA intake, DHA intake is not as clearly linked to lower risk of AD as is fish intake [98]. A precise quantitative estimate of DHA intake is generally impossible from food frequency questionnaires because the number of fish items they provide is too limited and commonly not adequately validated for this purpose. Moreover, EPA and DHA intake has high day-to-day variability because fish and seafood intake is typically sporadic. Random within-person error due to day-to-day fluctuation in dietary intake and measurement error tends to bias relative risks toward 1, i.e. to underestimate the strength of the possible association between the exposure (DHA intake) and the outcome (cognitive status) [99].

Moreover, consistency between fish and DHA intake in disease associations as such AD depends on the power of the study to detect an association, which itself is strongly linked to the total number of subjects involved (not only AD cases). In spite of appropriate adjustments, other residual confounders in observational studies usually remain: (i) Fish consumers differ from non-consumers in many ways which may themselves be associated with higher or lower risk of dementia e.g. education, income, lifestyle [91]. (ii) Fish is not eaten alone but is a component of “healthy” dietary pattern e.g. the Mediterranean diet itself associated with lower dementia risk [100]. Moreover, the recently identified interaction of AD risk with ApoE genotype requires stratification on ApoE4 status for a proper interpretation of the association between fish or DHA intake and dementia (see Section 6.2.2 - Apolipoprotein E).

5. BIOLOGICAL STUDIES OF DHA IN AD AND OTHER FORMS OF COGNITIVE DECLINE

5.1 Blood DHA

Lower blood DHA occurs in those consuming low amounts of fish while higher plasma DHA is observed in those consuming more fish [89, 101]. Indeed, lower blood DHA is observed in those adhering to a diet strictly avoiding fish or seafood, i.e. in vegans [102]. The amount of DHA in human blood is normally significantly positively correlated to DHA intake, but at DHA intakes >2 g/d, DHA in plasma phospholipids approaches saturation and increases only marginally thereafter [97]. Since AD and other forms of cognitive decline are significantly associated with low fish intake and lower DHA intake (Table 1), one would normally expect to see that AD was associated with lower plasma and erythrocyte DHA. We review here the evidence for this prediction from both prospective and cross-sectional studies.

5.1.1 Blood DHA - Prospective Studies

Five published prospective studies have reported plasma DHA in the elderly with cognitive decline after follow-up [103-105] or who converted to AD or other dementia [105, 106] (Table 2; Figure 1). Of these five prospective studies, only the one by Heude et al., [104] reported significantly lower plasma DHA. Therefore, a crucial point is that although mean plasma or erythrocyte DHA is not different from control values in most prospective studies, these studies nevertheless demonstrate a significant inverse association between baseline plasma or erythrocyte DHA and *risk of cognitive decline* or incident dementia [89, 103, 106, 107]. For instance, the largest prospective study to date was conducted in a subsample of the Three City study [106]. It showed that for each increase of one standard deviation in plasma DHA, risk of all-cause dementia was reduced by 24%. Nevertheless, mean blood DHA in those converting to AD compared to those that did not convert was similar in both groups. In

the Three City study [106], the association of higher blood DHA to lower risk of cognitive decline became non-significant in multivariate analyses, but the inverse association with plasma EPA remained.

The longest prospective study of the association between DHA intake, plasma DHA and cognitive decline in the elderly had a mean follow-up of 9.1 years. It reported that risk of all causes of dementia was significantly associated with both lower DHA intake and lower DHA in plasma phosphatidylcholine [89]. This study also showed a significantly reduced risk (~50%, $p=0.05$) for all-cause dementia in the highest quartile of plasma DHA [89]. However, the direct association between lower DHA intake and higher risk of all causes of dementia was present only when the lower three quartiles of DHA intake were combined and compared to the highest DHA intake. It was also only significant for all causes of dementia but not for AD alone. Apart from the large number of subjects studied, one of the strengths of this study was the assessment of DHA intake and plasma DHA in a specific class of blood phospholipids (phosphatidylcholine).

In the study by Beydoun et al. [103] which was based on the Atherosclerosis Risk in Communities study, higher plasma phospholipid or cholesteryl ester content of EPA and DHA was protective against decline in verbal fluency. However, no association was found with global cognitive decline or other neuropsychological tests in that study. In the placebo arm of the FACIT trial, higher long chain $\omega 3$ PUFA in plasma cholesteryl esters was associated with less cognitive decline over 3 years on tests of sensorimotor speed and complex speed but in contrast to Beydoun et al [103], was unrelated to changes in word fluency [107]. The largest prospective study reporting fatty acid analyses showed a significantly decreased risk of global cognitive decline with higher erythrocyte EPA, DHA and total $\omega 3$ fatty acids [104]. Hence, it is very important to make a distinction between static blood DHA measurements in those with or

without cognitive decline, versus the more dynamic measure of blood DHA *in relation to risk of cognitive decline*.

5.1.2 Blood DHA - Cross-sectional Studies

There are more published reports of blood DHA in dementia, AD or other forms of cognitive decline that are based on cross-sectional studies than there are based on prospective studies. Nevertheless, relative to each study's own controls, our *post hoc* analysis is that overall mean blood DHA values for AD or other forms of cognitive decline is still not different from 100%, i.e. not different from blood DHA in healthy age-matched controls (Figure 2). If one outlying cross-sectional study [108] is deleted from this analysis, overall blood DHA values in AD and other forms of dementia relative to controls is actually numerically (but not statistically) lower in these pooled cross-sectional studies than in the prospective studies (compare Figures 1, 2). The lack of association of low blood DHA with AD in cross-sectional studies may also be due in part to modification of dietary intake during progression towards dementia and the related difficulties that subsequently arise in activities of daily living such as shopping or preparing meals.

Three key points emerge from this *post hoc* analysis of the published literature: First, compared to control values, blood levels of DHA (plasma or erythrocytes) are not different in AD or other forms of dementia, regardless of whether the studies are prospective or cross-sectional in design (Table 2, Figures 1,2). Second, without reference to changing cognitive function (preferably in a prospective study design), blood DHA values alone are not a very useful measure of cognitive status. Third, it remains to be established whether the overall similarity in plasma and erythrocyte DHA levels seen in AD as in healthy age-matched controls is a function of masking due to genotype effects, other aspects of study design including the generally small numbers of subjects in most cross-sectional studies, or mixing of

data for cognitive decline of different etiology. Alternatively, they may also be a function of changes in DHA metabolism brought on by cognitive decline itself (see Section 6 - Linking Epidemiological and Biological Studies) [109].

5.2 Brain DHA

If low DHA intake increases the risk of deteriorating cognition in the elderly, to understand the link between dietary DHA and risk of declining cognition in the elderly, it is important to know whether low brain DHA occurs in those with clinical evidence of cognitive decline, particularly AD. Our analysis here is based on about 100 brain DHA analyses from AD patients and age-matched controls reported in seven independent publications (Table 3). For the hippocampus, the three published studies all showed AD brain had 30-50% lower free DHA and also lower DHA in brain PE. However, in the parahippocampus, brain DHA was not different in AD compared to healthy, age-matched controls. Barring the results of one study [110], overall values for DHA in frontal cortex phospholipids were variable but, on average, also very similar in AD compared to healthy controls (Table 3; Figure 3). Phospholipids are the brain's main repository of DHA but this pool may not be as important as free DHA could be in helping the brain resist the oxidative damage occurring in AD. Free DHA is more labile and although only one reports gives values for free DHA (in the hippocampus), they were 50% lower in AD than in controls [19] (Table 3; Figure 3).

However, when low brain DHA was observed, it was rarely specific for DHA: either ARA and/or adrenic acid (22:4 ω 6) were also decreased [110-112]. In some reports, lower DHA concentration was specifically due to a decrease in one of the brain phospholipid classes, most commonly plasmalogens but not in the % composition of DHA itself in the individual phospholipid classes [113]. In AD, increases of up to 30% in the DHA content of frontal cortex, superior and middle temporal and in the white matter of parietal and frontal

cortex have also been reported [113-115]. Four fold higher adrenic acid has been observed for the gray matter of parietal and frontal cortex and in the parahippocampal region [113-115]. Thus, there is considerable disparity and non-specificity for DHA data in AD brain. Indeed, there is actually more agreement about the importance of a significant decrease in the concentration of brain phospholipids in AD, particularly in plasmalogen PE, than there is about changes in brain DHA *per se* [109]. The changes in brain phospholipid (or free fatty acid) pool size during AD may be equally or more important than the possible changes in fatty acid composition of these lipid classes [116].

One difference between these studies that might help account for this disparity of brain DHA data is that different researchers have used different criteria to define their AD groups. PL and DHA content of brain tissue also varies markedly from region to region [117], especially from white to gray matter. Hence, imprecise dissection of brain tissue used in these analyses could also markedly skew the results. Nevertheless, one report shows lower DHA in both white and gray matter [110]. Possible differences in analytical methodology are not always easy to identify but could also contribute to variation in reported DHA values.

Aside from methodological issues, homeostatic mechanisms slow down brain DHA loss during dietary ω 3 PUFA deprivation [118], so this might affect the rate of decline of brain DHA in the elderly or in response to processes precipitating AD. Animal studies suggest that plasma DHA varies much more than brain DHA and is much more influenced by dietary ω 3 PUFA intake. Hence, even if mild, chronic dietary ω 3 PUFA (Table 1) might help the AD brain resist losing DHA, at least until the stage at which neurodegeneration and lipid peroxidation were extensive.

The natural atrophy of the human brain as it ages [119] adds a complication to expression of brain fatty acid data because it affects the point of reference, whether it be

tissue weight (dry or wet), phospholipid content or protein or DNA content (See Section 6.2.1 – DHA Metabolism During Healthy Aging). Nevertheless, in AD, DHA seems to be markedly lower in the hippocampus, a brain region intimately involved in memory formation and retrieval (Figure 3). It remains to be determined whether this relatively specific change in brain DHA is in part a function of inadequate DHA intake or specific pathological mechanisms more rapidly destroying DHA in the hippocampus than elsewhere in the brain.

5.3 Clinical Trials

Converging results from animal and observational studies show a protective effect of dietary consumption of fish or ω 3 PUFA for risk of AD but, to date, just a single randomized controlled trial (RCT) has been reported for the *primary prevention* of cognitive decline by EPA and DHA [120]. In that study, 302 cognitively healthy older participants were randomly assigned to receive either 1,800 mg/d EPA+DHA, 400 mg/d EPA+DHA, or placebo capsules for 26 weeks. There were no significant differential changes in any cognitive domain among the three groups at either 13 or 26 weeks intervention except slightly more decline in memory at 13 weeks in the 400 mg group than the placebo, a difference that might well be attributable to chance. An interaction with ApoE genotype was observed, i.e. ApoE4 carriers in both fish oil groups showed an improvement on the cognitive domain of attention after 26 weeks of intervention compared with placebo. However, such post-hoc analyses in small subgroups must be interpreted with caution, all the more since the interaction was not consistent with that found in observational studies [84, 86, 103].

Several RCTs have focused on patients who already suffered from various stages or types of dementia or cognitive impairment. To date, the largest, published double-blind RCT in mild to moderate AD recently showed very modest cognitive benefits of a mixed dietary PUFA supplement containing both DHA (1.7 g/g) and EPA (0.6 g/d) compared to a corn oil

placebo [121]. No overall difference in cognitive function assessed by the Mini-Mental Status Examination (MMSE) or the modified cognitive portion of the Alzheimer Disease Assessment Scale (ADAS-cog) was observed between the controls and the intervention group after the first 6 months of active or placebo treatment. However, in a sub-group of 32 AD patients who had a relatively high level of cognitive function at the start of the study, (MMSE >27), MMSE scores declined significantly more slowly during the first 6 months of active treatment [121]. Interestingly, cognitive decline seemed to halt when the placebo group also received the ω 3 supplementation during the 6 following months. Again, such post-hoc analyses must be interpreted with caution since the randomization was not stratified by cognitive level and could not therefore ensure that the placebo and intervention groups were still comparable.

Similar results were reported in a recent small RCT [122]. The placebo and the treatment groups were patients at varying stages of AD or cognitive impairment. They consumed a mono-therapy of 1080 mg of EPA and 720 mg of DHA or olive oil esters as the placebo for 6 months. ADAS-Cog test scores improved significantly in the EPA+DHA group compared to the placebo but only in those with mild cognitive impairment. This study also showed that higher % EPA in erythrocytes was significantly associated with better cognitive outcome, suggesting that DHA may not be the only ω 3 PUFA linked to better cognition [122].

In an exploratory preventive RCT [123], 49 healthy elderly women received a DHA supplement or placebo for 4 months with or without lutein. Multiple cognitive tests measuring verbal fluency, memory processing speed and accuracy were performed at randomization and 4 months later. DHA alone or in combination with lutein significantly improved verbal fluency, whereas rate of learning and delayed recall were improved only in the group receiving the DHA plus lutein supplement [123]. DHA supplementation caused no change on any other test. Higher DHA serum level was significantly associated with better verbal fluency.

However, this small trial had several limitations, including a high rate of dropouts not taken into account by an intention-to-treat analysis (14%), and an important ceiling effect on most cognitive tests which prevented any improvement.

Three other small preliminary trials of supplements combining DHA and EPA or DHA and ARA in patients with various forms of dementia have also been reported, and all three showed that the supplement had no clear benefit for cognitive function [121]. In Japan, a very small RCT on 20 patients suffering from dementia from thrombotic cerebrovascular disease showed an improvement in MMSE score with 720 mg DHA/d for 6 months [124]. Conversely, a small open-label pilot study on the efficacy of ethyl-EPA (500 mg twice daily over 12 wk) for the treatment of patients with mild to moderate AD did not demonstrate any effect on cognition [125]. Finally, a double-blind RCT that evaluated the impact of 1.5 g/d of EPA+DHA for 12 weeks in 190 mildly depressed individuals did not observe any benefit on mood or cognitive function [126].

Hence, at best, a very modest clinical benefit of mixtures of PUFA including DHA has been reported but it is not yet clear whether DHA itself has any effect on aging-associated cognitive decline or AD. Several aspects of the clinical trials with DHA or other PUFA will need further study before conclusions can be drawn about the efficacy of such dietary interventions to improve various features of cognition. First, the breadth of doses of DHA used in these studies covered a 36 fold range - 120 mg/d to 4.3 g/d (see Table 4), a huge range that, importantly, produced no apparent dose-related effects on cognition. Second, two of these clinical trials raised the possibility that a beneficial effect of supplemental DHA may exist when cognitive decline is mild but that supplemental DHA may not necessarily be recommended when the decline is more severe, possibly because additional DHA might contribute to degenerative processes in the brain related to lipid peroxidation [121, 122]. Third, only one of these studies [120] separated the groups according to ApoE4 genotype, a

factor which may well compromise the protective effect of dietary ω 3 PUFA in lowering the risk of progression towards serious forms of cognitive decline (see Section 6.2.2 - Apolipoprotein E).

6. LINKING EPIDEMIOLOGICAL AND BIOLOGICAL STUDIES

A link is starting to emerge between lower fish intake, lower DHA and heightened risk of AD (Table 1). However, our interpretation is that this link is still tentative. For instance, within the prospective studies, three studies tentatively linking estimated DHA intake from fish and seafood to lower risk for dementia and AD were not consistent (Table 1): The Zutphen study reported a protective effect of total DHA+EPA intake against cognitive decline [127] which was not found in the CHAP study (which only reported total ω 3 fatty acid intake) [88]. Conversely, the latter showed a protective effect of DHA intake specifically against AD without reporting about other causes of dementia [87]. In a subsample of the Framingham study, the opposite was observed, i.e. protection by DHA against all cause dementia but not AD [89]. Also, at least statistically, low fish intake seems to have a stronger association with risk of AD than does low DHA intake. Hence, it seems premature to conclude that the protective effect of fish against risk of AD can be equated to a protective role specifically of DHA.

The presumed link between lower fish (or DHA) intake and lower blood DHA in AD is also very tentative (Figures 1,2), in part because blood DHA data in AD have fairly wide dispersion. Brain DHA data for AD also vary considerably. Three studies do suggest that DHA may be lower in the hippocampus in AD (Figure 3), it is not clear that this effect is specific for DHA, either in the hippocampus or in other brain regions that have been studied. Currently, no studies link blood or brain DHA to the neuropathology specific to AD but such a

relationship would greatly strengthen a mechanistic link between low DHA and specific processes contributing to AD.

Hence, broadly, there are two possibilities: either there really is no biological link between risk of AD and low DHA intake, low blood or low brain DHA or, owing methodological or other experimental differences between studies, the moderately good link present in the epidemiological literature is simply not clearly revealed in the biological literature as it presently stands, perhaps because features of most of the studies mask or confound the variables under study. Some of the methodological challenges affecting the possible relationship between fish intake, DHA intake and risk of AD will be discussed first; biological reasons for the masking of such a possible relationship will be discussed subsequently (see Section 6.2 - Biological Issues).

6.1 Methodological Issues

The possible relationship between DHA intake and AD is vulnerable to the classical pitfalls of nutritional epidemiology affecting observational studies. One major limitation is that despite appropriate adjustments, residual confounders still contribute importantly to the variability observed. Higher DHA intake may also merely be a marker of a generally protective lifestyle including a healthy diet, cognitive and physical activities, and better management of vascular risk factors, all of which could contribute to maintain optimal brain functioning during aging but for which complete adjustment cannot be achieved.

Observational studies also face the difficulty of estimating true quantities of dietary intake of specific nutrients due to cumulative sources of variability and error, i.e. day-to-day intra-individual variability, reliability of food frequency questionnaires, dietary recall, and food composition tables. Most published reports have not considered cooking methods but concentrations of EPA and DHA in plasma phospholipids may correlate more with

consumption of non-fried but not with fried fish [95]. Moreover, it is impossible to differentiate the specific effects of a nutrient such as DHA from those of another one to which it is closely linked in the usual diet (see Section 6.2.3 - Other Nutrients in Fish). Some of the lack of agreement between observational studies of fish intake or DHA levels and those using DHA supplementation is potentially attributable to artifacts caused by methodological differences, including different definitions of cognitive function or dementia, different analytical methods, and possibly even different definitions of 'healthy' controls.

Intervention studies, even RCTs, have their own pitfalls. Contrary to RCTs with drugs, administration of nutritional supplements does not start from zero intake of the nutrient in the supplement. Indeed, individuals are already exposed to varying dietary sources of EPA and DHA, which may confound the true effect of the supplementation. Differing background DHA intakes, especially the very low DHA and EPA intakes in the USA, may also play a role in the lack of agreement. In principle, randomization should correct for different basal dietary EPA and/or DHA exposure in the intervention and control groups. Nevertheless, as the target population for an RCT, individuals may be selected who are more or less prone to suffer from insufficient dietary intake of the supplement to be used, e.g. hospitalized elderly who are at higher risk of undernutrition [124]. These individuals might benefit more from the supplementation than healthy community dwellers such as those included in a large primary prevention trial [120]. As already shown for RCT of fish oil supplementation in cardiovascular disease [26], overly optimistic estimates of effect sizes, poor patient adherence to a long course supplementation, and higher than anticipated losses to follow-up may all reduce the power of a trial and hence its ability to demonstrate significant differences.

Indeed, even the placebo can create bias; olive oil might have some protective effects against cognitive decline [128], while a placebo based on $\omega 6$ fatty acids such as linoleic acid might increase the risk of dementia [84]. The true difference between the intervention and

treatment groups would therefore be artificially minimized in the first case, but increased in the second. Of course, 240 mg olive oil is a very small quantity compared to the overall dietary intake of some populations but 1 g corn oil provides 0.6 g linoleic acid, which could contribute importantly to total intake of ω 6 fatty acids.

Another major limitation of most intervention studies is their poor selection of individuals who could actually benefit from a dietary supplement because of their low spontaneous DHA intake. In the clinical trials with DHA, different time courses and different durations of DHA supplementation or composition of the supplements make the results difficult to interpret. The duration of the supplementation is generally limited to a few weeks or months, which may be quite insufficient to reverse a chronic deficiency and the associated cognitive dysfunction despite the rapid plateau in plasma levels achieved in approximately one month after supplementation [101].

6.2 Biological Issues

If there really is a link between higher intake of DHA itself (or other ω 3 fatty acids) and lower risk of AD, then further research is needed to identify and understand the biological processes that could mask this relationship and prevent the normal link between low DHA intake and low plasma DHA from being seen in AD. DHA synthesis and β -oxidation are both very low in humans [89] so, unlike in many animal models, in non-vegan humans, DHA intake has a very important effect on whole body DHA homeostasis [97]. To our knowledge, no study has yet examined the association of DHA and AD in vegans or other populations that strictly eat no fish. The brain is rich in DHA and brain DHA levels are relatively hard to manipulate after weaning, so it is somewhat more understandable that brain DHA levels could be the same in AD as in healthy, age-matched controls. Lipid peroxidation and other

degenerative processes linked to inflammation that play a key role in AD may degrade and deplete DHA but their quantitative impact on DHA levels would be small unless lipid peroxidation was occurring on a massive scale. If lipid peroxidation did consume brain DHA, one would expect DHA levels to decrease but not more so than the other long chain PUFA in the brain (mostly ARA and adrenic acid).

Depression also is a factor that may contribute to AD, and two prospective studies have reported higher plasma ARA:DHA in more depressed subjects [107]. Interestingly, higher plasma phospholipid EPA and DHA was protective against decline in verbal fluency but only in the least depressed subjects; no such association was found with global cognitive decline or other tests in that study [104].

At DHA intakes up to 2 g/d, plasma phospholipid DHA concentrations increase in a dose-dependent, saturable manner in response to dietary DHA [101]. Red blood cell kinetic follows a similar pattern, but it takes a longer time to reach a steady state [101]. Peripheral DHA levels, in particular in plasma, may not adequately reflect DHA incorporation into neuron membranes. Red blood cells might be a better compartment to assess the link between habitual DHA intake and brain DHA. On the other hand, plasma DHA may be a more sensitive measure of short term dietary intake and release from membrane phospholipids after phospholipase cleavage. Plasma DHA might therefore better reflect the bioavailability of fatty acids as substrates for cyclooxygenases and lipoxygenases but be poor predictors of the long-term effect of dietary DHA on brain aging.

Rapoport [129] estimates that the healthy young adult human brain turns over DHA at a rate of about 4.5 mg/d. If Rapoport's calculation is correct, why the necessary daily dose of DHA to impede cognitive decline in AD varies so widely (from as little as 180 mg/d to almost 40 times that amount; Table 4) also needs to be understood. The turnover of DHA comprises its use for membrane replacement, β -oxidation, synaptic integrity, gene regulation and

synthesis of docosanoids and neuroprotectins but does not take into account factors such as DHA losses to lipid peroxidation which could contribute to a higher DHA requirement during aging or, especially, in AD [130]. Moreover, the amount of DHA that should be consumed to meet the brain's requirement DHA is still not known.

Whether brain DHA turnover changes with healthy aging, presence of an ApoE4 allele or during cognitive decline remains to be determined. Whole body β -oxidation of a single dose of ^{13}C -DHA in healthy, young adults is very low (<5% in a one month follow-up; [131]), suggesting that, like the brain, the healthy, adult human body is not rapidly catabolizing large amounts of DHA relative to average DHA intakes common in North America (generally ≥ 100 mg/d; [132, 133]). Therefore, a better understanding of DHA metabolism, including its transport and β -oxidation seems crucial to better assess the changes occurring during healthy aging and during cognitive decline.

6.2.1. DHA Metabolism During Healthy Aging

There are no widely recognized criteria for healthy aging or healthy elderly controls. In research studies, mean ages of elderly groups can be as low as 50 y or as high as 75-80 y old. Identifying changes in $\omega 3$ PUFA metabolism that occur during healthy aging may help to determine how dietary DHA or EPA contributes to healthy aging and, by inference, how they might reduce the risk of cognitive decline and AD.

We compared the plasma DHA response to a fish oil supplement in healthy elderly and young adults [134]. Our subjects were screened for clinical evidence of cardiovascular disease, chronic inflammation, thyroid, liver and renal disease, malnutrition, and even early indicators of diabetes. At baseline, both EPA and ARA were higher in elderly compared to young adults. During the fish oil supplementation phase, plasma DHA increased significantly

higher and more rapidly in the elderly, reaching a plateau after 2 weeks of supplementation whereas in young, plasma DHA was still increasing after 3 weeks [134]. EPA and ARA remained higher in elderly compared to young throughout the fish oil supplementation. Other reports confirm the likelihood that baseline fasting plasma EPA and DHA are somewhat higher in the healthy elderly [135-137]. These results suggest that long chain PUFA metabolism is subtly altered during healthy aging, due possibly to changes in their β -oxidation or perhaps to lower capacity of organs such as the brain to utilize EPA, DHA and ARA.

We have also unpublished results using a unique tracer – uniformly carbon-13 (^{13}C)-labeled DHA - in young and elderly. Our preliminary results suggest that the incorporation of ^{13}C -DHA into plasma TG and free fatty acids is already different between young and healthy elderly subjects within the first four hours after its intake. In a follow-up of one month, overall β -oxidation of ^{13}C -DHA was similar between young and elderly but four hours after the single oral dose of ^{13}C -DHA, β -oxidation was significantly higher in the elderly, an effect corresponding possibly to the higher plasma incorporation of ^{13}C -DHA 4 hours dosing. Therefore, our data suggest that ω 3 PUFA metabolism changes mildly but significantly during healthy aging. Whether this would have any impact on risk of cognitive decline is still unknown.

Not only do plasma ω 3 PUFA levels and response to a supplement change during healthy aging, but the brain also seems to undergo important changes in lipid composition. Between 20 y old and 100 y old, the healthy brain loses 20% of its weight, 36% of its dry matter weight [119]. Lipid content is markedly affected by the brain atrophy associated with aging since cholesterol and phospholipids decrease by 47% and 42%, respectively [119]. Unfortunately, brain PUFA composition was not reported in this study. Nevertheless, it is clear

that better criteria to define healthy elderly controls are needed, which may avoid some of the conflicting data reported in relation to PUFA and cognitive deterioration in the elderly.

6.2.2 Apolipoprotein E

One of the biological factors that may mask the relationship between DHA and lower risk for AD is polymorphisms in ApoE. Around 23% of the Caucasian population carries, i.e. has at least one copy, of the ϵ 4 allele of ApoE [138], which is the genetic risk factor most associated with late onset or sporadic AD. Premature cognitive decline in ApoE4 carriers occurs in midlife, a decade or more before the onset of MCI and/or AD [139]. Children aged 11 years old with higher general intelligence had higher erythrocyte ω 3 PUFA, but the correlation was only significant for those without the ϵ 4 allele of ApoE [140]. The same population was retested at 64 years old and their general intelligence was even more highly positively correlated with erythrocyte ω 3 PUFA, but there was still no correlation in carriers of ApoE4 [140].

Two recent prospective studies indicate that ApoE4 carriers are not protected against dementia by higher fish intake [84, 86]. Nevertheless, one recent study evaluating the impact of fish oil on cognitive performance in the healthy elderly reported that after 26 weeks of supplementation with either 0.4 or 1.8 g/d of ω 3 PUFA, ApoE4 carriers had improved scores on tests of the cognitive domain of attention compared to the placebo [120]. Plasma EPA and DHA concentrations were reported but no information was provided as to whether they changed differentially in relation to ApoE4 genotype.

Response of plasma DHA and plasma lipids to a fish oil supplement is influenced by the ApoE4 genotype [141-143]. We have preliminary results on incorporation of EPA and DHA into plasma lipids of ApoE4 carriers and non-carriers that clearly support this gene-by-

diet interaction [144]. We analyzed the plasma fatty acid profiles of 8 carriers and 20 non-carriers of ApoE4, before and after 6 wk of 3 g/d of EPA and DHA combined [144]. In contrast to what we anticipated at baseline, ApoE4 carriers had significantly *higher* EPA and DHA in plasma TG. Unlike in non-carriers of ApoE4, EPA in plasma free fatty acids in ApoE4 carriers did not change nor did DHA change in plasma TG after the supplement. These recent data suggest that the ApoE4 interaction with PUFA metabolism is a nutrient-gene interaction which must be taken into account when assessing the risk for AD.

In AD patients in the OmegAD study, weight gain and appetite improved in EPA+DHA group but only in those not carrying the ApoE4 allele [145]. Hence, DHA and/or EPA supplementation might also work through mechanisms that could contribute towards improved nutritional status of AD patients. Here again, the interaction with genetic polymorphisms deserves further research.

6.2.3. Other Nutrients in Fish

If there really is no link between low DHA and risk of AD but there is a link to low fish intake, other nutrients in fish that are currently drawing less attention than ω 3 PUFA may actually be the nutrients protective against AD. For instance, intake of fish protein may be protective against type 2 diabetes. In lean fish, protein is the most abundant nutrient [146]. Cod protein may affect glucose metabolism by improving insulin sensitivity [147] and may also lower plasma C-reactive protein [147] in insulin resistant men and women. Since much epidemiological evidence suggests that type 2 diabetes is associated with a 2-3 fold increased relative risk for AD [148, 149] and since aging itself is linked to somewhat increased prevalence of insulin resistance, improving insulin sensitivity by intake of fish protein may help in the management of whole body glucose metabolism [149]. Conversely, in

addition to ω 3 PUFA, fish oil capsules may provide some protective lipid-soluble vitamins but contain no fish protein.

The role of EPA (also found in fatty fish) in brain aging may have been underestimated. Indeed, higher plasma EPA but not DHA was strongly linked to lower risk of incident dementia in the Three City study [106]. The EPA:DHA ratio varies considerably across fish species. Whereas most fishes contain about twice as much DHA as EPA, the reverse is observed in Atlantic herring or shrimp [150]. Most studies used combinations of EPA and DHA which makes it difficult to discern specific roles and cognitive benefits of the individual ω 3 PUFA [101].

Other micro-nutrients found in fish may also exert protective effects on the brain, i.e. selenium, vitamins A and D, or vitamin B12 [94]. Plasma selenium concentration has been reported to correlate to that of plasma EPA and DHA [151]. In addition to long chain ω 3 PUFA, these nutrients might exert protective effects in the brain by various mechanisms, including the anti-oxidant properties of selenium, activation of vitamin D receptors and retinoic acid receptors in the brain by vitamins D [152] and A [153], respectively, and homocysteine-lowering effects of vitamin B₁₂ [154]. It is therefore very difficult to disentangle their respective effects. These nutrients might act as confounders in the relationship between fish intake and risk of AD, i.e. they would explain a protective effect wrongly attributed to DHA. Alternatively, they may also have synergistic effects which would enhance the protective action of DHA against brain aging. For instance, selenium is an anti-oxidant that may help protect DHA against lipid peroxidation. Similarly, DHA activates PPARs, which are a partner of dimerisation of RXR retinoid receptors activated by vitamin A derivatives [155].

Supplements of ω 3 PUFA could therefore be more efficacious if they included such synergistic nutrients. In that sense, the positive impact on several cognitive outcomes of a

supplement of DHA and lutein, a carotenoid, deserves further attention [123]. Indeed, some fish species such as salmon eat small invertebrates rich in carotenoid pigments which give them their color, and which are precursors of vitamin A.

6.2.4 Fish as a Component of a Healthier Diet

It may also be important to think in terms of dietary patterns rather than just the intake of specific nutrients or food types. For instance, in the Three City Study, when considered alone, fish intake was not significantly associated with decreased risk of dementia but this association was present when fish was jointly considered with daily consumption of fruits and vegetables [84]. Fish consumers are generally higher consumers of fruits and vegetables which provide antioxidants that help minimize the risk of lipid peroxidation [91] of long-chain PUFA. The DHA content of fish species may also vary considerably according to where they are caught or fish farming conditions [94].

Fish consumption is therefore widely viewed as a component of a healthy diet, one that also includes higher fruit and vegetable consumption that provide vitamin C, carotenoids, and polyphenols exerting potentially important anti-oxidant effects which could contribute to slowing down brain aging [91, 156-158]. Fish consumption is also a component of a Mediterranean dietary pattern whose protective effect against AD was recently described [100]. The combination of both long-chain ω 3 PUFA and anti-oxidants which will protect them against peroxidation could therefore be necessary.

6.2.5 Insufficient DHA or Excessive ω 6 PUFA?

DHA status should not be considered independently of ω 6 PUFA intake. Indeed, DHA and EPA compete with ARA for the sn-2 position on membrane phospholipids [101]. Is AD

associated with low DHA intake or is it rather a question of too much ω 6 PUFA intake? In the Three City study, higher plasma ARA:DHA was associated with increased risk of incident dementia whereas plasma concentrations of ARA or DHA alone were not [106]. Normal individuals could therefore potentially have a cognitive benefit from high intake of DHA and/or EPA but only if they correct an excessive intake of ω 6 fatty acids [159]. A dietary trial in which healthy adult men consumed a ratio of linoleic acid to alpha-linolenic acid of 4:1 or 10:1 showed that lowering linoleic acid intake was associated with higher plasma phospholipid EPA and lower plasma ARA:EPA, but did not modify ARA or DHA [160].

7. ANIMAL STUDIES

The transgenic mouse - Tg2576 - with the APP^{swe} mutation is the main animal model used to study the dietary effects of ω 3 PUFA on AD. These mice exhibit memory loss and A β deposition. In two studies, adult Tg2576 mice were given different levels of dietary ω 3 PUFA (control diet, ω 3 deficient diet, and the same ω 3 deficient diet supplemented with DHA) for about 100 days [161, 162]. ω 3 PUFA deficiency leads to a loss of key pre- and postsynaptic markers essential for cognition. Indeed, ω 3 PUFA deficiency decreased N-methyl-D-aspartate receptor subunits (pre-synaptic) in the cortex and hippocampus of mice and also decreased Ca²⁺/calmodulin-dependent protein kinase (postsynaptic) in the cortex. These effects were significantly higher in Tg2576 mice compared to the non-transgenic controls [161].

Reduction of dietary ω 3 PUFA resulted in a 80-90% decrease of the p85 α subunit of phosphatidylinositol 3-kinase and the post-synaptic actin-regulating protein, drebrin, a change also observed in the brains of AD patients. ω 3 PUFA deficiency also increased caspase-cleaved actin content and oxidative stress markers in the brain cortex [162]. Conversely, mice fed a ω 3 deficient diet supplemented with DHA were partly protected against these effects

and behavioral deficits. DHA-supplemented mice also exhibited improved spatial memory [161, 162]. A 40% decrease of the number and burden of amyloid plaques was observed in the same transgenic mouse model submitted to a high DHA diet (containing 0.6% DHA) compared to a low DHA diet ($\omega 6:\omega 3$ of 85:1, with 6% fat as safflower oil), but not control diet ($\omega 3:\omega 6$ of 7:1). DHA treatment also lowered the levels of A β 42 but not A β 40 in the cortex of these animals [163].

Conversely, in a double transgenic mouse model of AD expressing mutant genes for amyloid precursor protein and presenilin 1, a diet containing 4% fish oil with DHA as 5.7% of total fatty acids did not modify the hippocampal levels of either soluble or insoluble A β compared to mice receiving a standard control diet [159]. In that study, the high $\omega 3$ PUFA diet did not improve or protect the cognitive performance of the transgenic compared to non-transgenic mice. Brain levels of $\omega 6$ PUFA were strongly positively correlated with cognitive impairment, whereas no correlations existed between brain $\omega 3$ PUFA levels and cognitive performance in transgenic or non transgenic mice [159]. However, the small number of animals in this experiment ($n = 8$ in each group) may have hampered detection of a significant difference despite the decreases of 25% and 17%, respectively, in soluble A β 40 and A β 42 in the group receiving the DHA-enriched diet.

More recently, in the Tg-AD triple transgenic mouse model of AD that exhibits both A β and tau pathologies, soluble A β 40 and A β 42 levels were significantly reduced after 6 months of dietary supplementation with DHA. After 9 months of supplementation, the DHA diet significantly reduced levels of soluble A β 40 but not A β 42. Insoluble A β levels were unchanged by the diet. DHA also reduced the accumulation of tau and phosphorylated tau in the soluble fractions of protein extracted from the whole brain [164].

Other studies using non-transgenic animal models seem to confirm the protective effect of DHA on AD. Indeed, in a model of cognitive impairment in which the rats received an intracerebral infusion of A β 40, dietary DHA provided a beneficial effect against the impairment of learning ability [56, 165, 166]. In these studies, DHA also decreased A β 40 accumulation after its infusion. Some but not all animal studies suggest that dietary ω 3 PUFA depletion could potentiate markers of AD in the rodent brain, i.e. such as A β accumulation, amyloid plaques and cognitive impairment, whereas DHA seems to have a beneficial effect on some of these markers. Nevertheless, some inconsistencies exist between studies, including differences in the effect of DHA on the levels of A β and the impact of ω 3 PUFA supplementation on cognitive performances. Furthermore, few effects of DHA supplementation on tests of cognitive performance or behaviour have been reported in these AD models.

A good animal model of AD is of primary importance as it permits investigation of mechanisms of the pathology which cannot be explored in humans, even if these animals incompletely express clinical symptoms or markers of AD neuropathology. One lesser known animal model of AD is the primate, *Microcebus Murinus* (mouse lemur), which naturally exhibit age-related brain changes closer to those in humans than presently seen in rodent models. Neurodegeneration in the aged mouse lemur is characterized by brain atrophy, presence of amyloid plaques, cytoskeletal tau pathology and a loss of cholinergic neurons. Aged mouse lemurs lose their cognitive and social capacities and exhibit certain age-associated similarities with human AD, while animals with elderly normal brain aging maintain memory function and social interaction [167].

A major limitation of human studies is their inability to have access to the brain. Moreover, post-mortem studies are based on small and highly selected clinical series. Animal

studies are therefore complementary approaches to verify the hypotheses on the mechanisms of action of fatty acids in animal brains.

8. BRAIN GLUCOSE UPTAKE: AN EMERGING MECHANISM

Assuming that the difficulty linking plasma or brain DHA to cognitive decline in the elderly is mostly methodological, i.e. that higher fish intake really does reduce the risk of AD or other forms of cognitive decline in the elderly, the mechanism of such an important effect still needs to be defined. An overview of the biological roles of DHA that have been well established during the past 30 years is given in the section - Biological Functions of DHA; each of these roles could in some way be implicated in protecting the brain against AD. We add here a mechanism that is emerging as potential link between low DHA and risk of cognitive decline in the elderly – that of control of brain glucose uptake.

During healthy aging, brain glucose uptake decreases significantly in specific cortical regions [168], an effect that is more pronounced in the elderly with deteriorating cognitive function such as AD [169]. Experimental data support the hypothesis that ω 3 PUFA intake could affect neuronal activity by altering brain fuel supply and hence energy metabolism. The main energy requirement of the brain is for Na^+/K^+ pump activity in nerve terminals and for oxidative phosphorylation, both of which are decreased in ω 3 PUFA deficient rats [170, 171]. Glucose utilization, glucose transporter 1 (GLUT1) immunostaining and the amount of the endothelial glucose transporter GLUT1 protein are all decreased in the cerebral cortex in a rat model of dietary ω 3 PUFA deficiency characterized by lower brain DHA content [171, 172]. GLUT1 is located in endothelial cells of brain microvessels and at the endfoot processes of astrocytes and is therefore the gateway for glucose entry into the brain. In a more recent and preliminary study, rats fed a DHA-supplemented diet had increased GLUT1 expression [173].

These data suggest a possible role of DHA in the control of GLUT1 expression and glucose transport into the brain.

This emerging role of ω 3 PUFA and/or DHA in brain energy metabolism could be linked to brain glucose hypometabolism, which seems to be the earliest identified change in AD, occurring 25-20 years before the clinical horizon [174]. It is therefore now possible to address the questions: could DHA promote brain glucose metabolism in the elderly, and could ω 3 PUFA intake be a useful dietary approach to correcting impaired cerebral glucose metabolism during aging thereby reducing the risk of aging-related cognitive decline and AD?

9. DISCUSSION

AD has a major impact on the healthcare system and has no widely accepted treatment. Developing effective strategies to reduce its risk and possibly improve its treatment is therefore a matter of high importance. The effectiveness of strategies involving DHA to reduce the risk of AD depends on a good understanding of how low intake or tissue levels of DHA would increase the risk of AD or other forms of cognitive decline in the elderly. Since the extensive report of Maclean et al in 2005 [175], numerous important contributions have been made in this field. A fairly solid basis now exists for believing that low fish and DHA intake contribute significantly to the risk of AD. Lower blood DHA is also fairly convincingly linked to a higher *risk of cognitive decline* and AD. However, the overall literature on static measurements of either blood or brain DHA shows no statistical difference in AD compared to healthy controls. Thus blood DHA needs to be assessed in relation to cognitive status and, by itself, is not presently a very useful marker actual or impending cognitive decline. Clinical trials published to date that involve DHA supplementation have also not been that promising. Hence, we agree with the conclusion of Maclean et al [175] that more research is definitely

needed. However, we would suggest that the focus could be less on whether low dietary DHA is a factor increasing the risk of cognitive decline or AD and could be more on establishing *how* DHA would be biologically linked to AD.

Such mechanistic research is essential if DHA or other PUFA are to find a useful role as nutritional supplements protecting against risk of AD or other forms of dementia, well designed RCTs are urgently needed that target individuals with low dietary DHA intake and raised risk of cognitive decline but who are not yet demented. It is not yet clear whether dietary DHA only has a preventive role, i.e. in risk reduction, or whether it could also be used to treat AD; if the latter, how early DHA supplements should be started is uncertain. There is also a need to establish which categories of cognitive decline are likely to be responsive to higher intake of fish or DHA or other PUFA; i.e. AD, vascular dementia, mixed or other forms. Consensus is also needed on definitions of AD and criteria to be used for subject selection so that independent studies can be compared more directly. The optimal duration of supplementation still needs to be defined, i.e. not too short so as to be inconclusive, as do doses and an optimal EPA:DHA ratio that are effective but do not increase risk of brain damage due to lipid peroxidation.

In the absence of easily accessible and specific biomarkers of AD, exploratory trials are needed to assess the impact of various doses and proportions of EPA and DHA on intermediate biomarkers i.e. oxidative stress, inflammation, and A β concentration in older persons. Animal models may be able to help identify both mechanistic aspects of PUFA involvement in neurodegeneration and suitable biological markers of incipient cognitive decline.

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References

- [1] Blennow K, de Leon MJ, and Zetterberg H, Alzheimer's disease. *Lancet* 2006;368:387-403.
- [2] Boudrault C, Bazinet RP, and Ma DW, Experimental models and mechanisms underlying the protective effects of n-3 polyunsaturated fatty acids in Alzheimer's disease. *J Nutr Biochem* 2009;20:1-10.
- [3] Schmitz G, and Ecker J, The opposing effects of n-3 and n-6 fatty acids. *Prog Lipid Res* 2008;47:147-155.
- [4] American Psychiatric Association. 1994. Diagnostic and statistical manual of mental disorders, 4th ed. 4th ed. APA Press, Washington, DC.
- [5] Dubois B, Feldman HH, Jacova C, Dekosky ST, Barberger-Gateau P, Cummings J, Delacourte A, Galasko D, Gauthier S, Jicha G, Meguro K, O'Brien J, Pasquier F, Robert P, Rossor M, Salloway S, Stern Y, Visser PJ, and Scheltens P, Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. *Lancet Neurol* 2007;6:734-746.
- [6] Gauthier S, Reisberg B, Zaudig M, Petersen RC, Ritchie K, Broich K, Belleville S, Brodaty H, Bennett D, Chertkow H, Cummings JL, de Leon M, Feldman H, Ganguli M, Hampel H, Scheltens P, Tierney MC, Whitehouse P, and Winblad B, Mild cognitive impairment. *Lancet* 2006;367:1262-1270.
- [7] Petersen RC, Doody R, Kurz A, Mohs RC, Morris JC, Rabins PV, Ritchie K, Rossor M, Thal L, and Winblad B, Current concepts in mild cognitive impairment. *Arch Neurol* 2001;58:1985-1992.

- [8] Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, and Kokmen E, Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol* 1999;56:303-308.
- [9] Winblad B, Palmer K, Kivipelto M, Jelic V, Fratiglioni L, Wahlund LO, Nordberg A, Backman L, Albert M, Almkvist O, Arai H, Basun H, Blennow K, de Leon M, DeCarli C, Erkinjuntti T, Giacobini E, Graff C, Hardy J, Jack C, Jorm A, Ritchie K, van Duijn C, Visser P, and Petersen RC, Mild cognitive impairment--beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. *J Intern Med* 2004;256:240-246.
- [10] Hardy J, and Selkoe DJ, The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science* 2002;297:353-356.
- [11] McLean CA, Cherny RA, Fraser FW, Fuller SJ, Smith MJ, Beyreuther K, Bush AI, and Masters CL, Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Ann Neurol* 1999;46:860-866.
- [12] Meyer MR, Tschanz JT, Norton MC, Welsh-Bohmer KA, Steffens DC, Wyse BW, and Breitner JCS, APOE genotype predicts when [mdash] not whether [mdash] one is predisposed to develop Alzheimer disease. *Nat Genet* 1998;19:321.
- [13] Gillette Guyonnet S, Abellan Van Kan G, Andrieu S, Barberger Gateau P, Berr C, Bonnefoy M, Dartigues JF, de Groot L, Ferry M, Galan P, Hercberg S, Jeandel C, Morris MC, Nourhashemi F, Payette H, Poulain JP, Portet F, Roussel AM, Ritz P, Rolland Y, and Vellas B, IANA task force on nutrition and cognitive decline with aging. *J Nutr Health Aging* 2007;11:132-152.

- [14] Gomez-Pinilla F, Brain foods: the effects of nutrients on brain function. *Nat Rev Neurosci* 2008;9:568.
- [15] Luchsinger JA, and Mayeux R, Dietary factors and Alzheimer's disease. *Lancet Neurol* 2004;3:579-587.
- [16] Crawford MA, Docosahexaenoic acid in neural signaling systems. *Nutr Health* 2006;18:263-276.
- [17] Innis SM, Dietary (n-3) fatty acids and brain development. *J Nutr* 2007;137:855-859.
- [18] Calon F, and Cole G, Neuroprotective action of omega-3 polyunsaturated fatty acids against neurodegenerative diseases: evidence from animal studies. *Prostaglandins Leukot Essent Fatty Acids* 2007;77:287-293.
- [19] Lukiw WJ, Cui JG, Marcheselli VL, Bodker M, Botkjaer A, Gotlinger K, Serhan CN, and Bazan NG, A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease. *J Clin Invest* 2005;115:2774-2783.
- [20] Florent S, Malaplate-Armand C, Youssef I, Kriem B, Koziel V, Escanye MC, Fifre A, Sponne I, Leininger-Muller B, Olivier JL, Pillot T, and Oster T, Docosahexaenoic acid prevents neuronal apoptosis induced by soluble amyloid-beta oligomers. *J Neurochem* 2006;96:385-395.
- [21] Bate C, Marshall V, Colombo L, Diomedea L, Salmona M, and Williams A, Docosahexaenoic and eicosapentaenoic acids increase neuronal death in response to HuPrP82-146 and A[beta]1-42. *Neuropharmacology* 2008;54:934.

- [22] Kawarabayashi T, and Shoji M, Plasma biomarkers of Alzheimer's disease. *Curr Opin Psychiatry* 2008;21:260-267.
- [23] Solfrizzi V, D'Introno A, Colacicco AM, Capurso C, Todarello O, Pellicani V, Capurso SA, Pietrarossa G, Santamato V, Capurso A, and Panza F, Circulating biomarkers of cognitive decline and dementia. *Clin Chim Acta* 2006;364:91-112.
- [24] Balk EM, Horsley TA, Newberry SJ, Lichtenstein AH, Yetley EA, Schachter HM, Moher D, MacLean CH, and Lau J, A collaborative effort to apply the evidence-based review process to the field of nutrition: challenges, benefits, and lessons learned. *Am J Clin Nutr* 2007;85:1448-1456.
- [25] Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. *Lancet* 1999;354:447-455.
- [26] Leon H, Shibata MC, Sivakumaran S, Dorgan M, Chatterley T, and Tsuyuki RT, Effect of fish oil on arrhythmias and mortality: systematic review. *Bmj* 2008;337:a2931.
- [27] Tavazzi L, Maggioni AP, Marchioli R, Barlera S, Franzosi MG, Latini R, Lucci D, Nicolosi GL, Porcu M, and Tognoni G, Effect of n-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): a randomised, double-blind, placebo-controlled trial. *Lancet* 2008;372:1223-1230.
- [28] Yokoyama M, Origasa H, Matsuzaki M, Matsuzawa Y, Saito Y, Ishikawa Y, Oikawa S, Sasaki J, Hishida H, Itakura H, Kita T, Kitabatake A, Nakaya N, Sakata T, Shimada K, and Shirato K, Effects of eicosapentaenoic acid on major coronary events in

hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. Lancet 2007;369:1090-1098.

[29] Yaffe K, Metabolic syndrome and cognitive disorders - Is the sum greater than its parts? Alzheimer Disease and Associated Disorders 2007;21:167-171.

[30] Kalmijn S, Foley D, White L, Burchfiel CM, Curb JD, Petrovitch H, Ross GW, Havlik RJ, and Launer LJ, Metabolic cardiovascular syndrome and risk of dementia in Japanese-American elderly men. The Honolulu-Asia aging study. Arterioscler Thromb Vasc Biol 2000;20:2255-2260.

[31] Raffaitin C, Gin H, Empana JP, Helmer C, Berr C, Tzourio C, Portet F, Dartigues JF, Alperovitch A, and Barberger-Gateau P, Metabolic syndrome and risk for incident Alzheimer's disease or vascular dementia: the Three-City Study. Diabetes Care 2009;32:169-174.

[32] Caslake MJ, Miles EA, Kofler BM, Lietz G, Curtis P, Armah CK, Kimber AC, Grew JP, Farrell L, Stannard J, Napper FL, Sala-Vila A, West AL, Mathers JC, Packard C, Williams CM, Calder PC, and Minihane AM, Effect of sex and genotype on cardiovascular biomarker response to fish oils: the FINGEN Study. Am J Clin Nutr 2008;88:618-629.

[33] Griffin MD, Sanders TAB, Davies IG, Morgan LM, Millward DJ, Lewis F, Slaughter S, Cooper JA, Miller GJ, and Griffin BA, Effects of altering the ratio of dietary n-6 to n-3 fatty acids on insulin sensitivity, lipoprotein size, and postprandial lipemia in men and postmenopausal women aged 45-70 y: the OPTILIP Study. Am J Clin Nutr 2006;84:1290-1298.

- [34] Gunnarsdottir I, Tomasson H, Kiely M, Martinez JA, Bandarra NM, Morais MG, and Thorsdottir I, Inclusion of fish or fish oil in weight-loss diets for young adults: effects on blood lipids. *Int J Obes (Lond)* 2008;32:1105.
- [35] Kelley DS, Siegel D, Vemuri M, and Mackey BE, Docosahexaenoic acid supplementation improves fasting and postprandial lipid profiles in hypertriglyceridemic men. *Am J Clin Nutr* 2007;86:324-333.
- [36] Krebs JD, Browning LM, McLean NK, Rothwell JL, Mishra GD, Moore CS, and Jebb SA, Additive benefits of long-chain n-3 polyunsaturated fatty acids and weight-loss in the management of cardiovascular disease risk in overweight hyperinsulinaemic women. *Int J Obes (Lond)* 2006;30:1535.
- [37] Maki KC, Van Elswyk ME, McCarthy D, Hess SP, Veith PE, Bell M, Subbaiah P, and Davidson MH, Lipid responses to a dietary docosahexaenoic acid supplement in men and women with below average levels of high density lipoprotein cholesterol. *J Am Coll Nutr* 2005;24:189-199.
- [38] Nagao K, and Yanagita T, Bioactive lipids in metabolic syndrome. *Prog Lipid Res* 2008;47:127.
- [39] Schwellenbach LJ, Olson KL, McConnell KJ, Stolcpart RS, Nash JD, and Merenich JA, The triglyceride-lowering effects of a modest dose of docosahexaenoic acid alone versus in combination with low dose eicosapentaenoic acid in patients with coronary artery disease and elevated triglycerides. *J Am Coll Nutr* 2006;25:480-485.

- [40] Stark KD, Park EJ, Maines VA, and Holub BJ, Effect of a fish-oil concentrate on serum lipids in postmenopausal women receiving and not receiving hormone replacement therapy in a placebo-controlled, double-blind trial. *Am J Clin Nutr* 2000;72:389-394.
- [41] Thies F, Garry JMC, Yaqoob P, Rerkasem K, Williams J, Shearman CP, Gallagher PJ, Calder PC, and Grimble RF, Association of n-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: a randomised controlled trial. *Lancet* 2003;361:477.
- [42] Florent-Bechard S, Malaplate-Armand C, Koziel V, Kriem B, Olivier JL, Pillot T, and Oster T, Towards a nutritional approach for prevention of Alzheimer's disease: Biochemical and cellular aspects. *J Neurol Sci* 2007.
- [43] Floyd RA, Neuroinflammatory processes are important in neurodegenerative diseases: an hypothesis to explain the increased formation of reactive oxygen and nitrogen species as major factors involved in neurodegenerative disease development. *Free Radic Biol Med* 1999;26:1346-1355.
- [44] Cunningham C, Campion S, Lunnon K, Murray CL, Woods JF, Deacon RM, Rawlins JN, and Perry VH, Systemic inflammation induces acute behavioral and cognitive changes and accelerates neurodegenerative disease. *Biol Psychiatry* 2009;65:304-312.
- [45] Bazan NG, Omega-3 fatty acids, pro-inflammatory signaling and neuroprotection. *Curr Opin Clin Nutr Metab Care* 2007;10:136-141.
- [46] Himmelfarb J, Phinney S, Ikizler TA, Kane J, McMonagle E, and Miller G, Gamma-tocopherol and docosahexaenoic acid decrease inflammation in dialysis patients. *J Ren Nutr* 2007;17:296-304.

- [47] Mori TA, Woodman RJ, Burke V, Puddey IB, Croft KD, and Beilin LJ, Effect of eicosapentaenoic acid and docosahexaenoic acid on oxidative stress and inflammatory markers in treated-hypertensive type 2 diabetic subjects. *Free Radic Biol Med* 2003;35:772-781.
- [48] Murphy KJ, Meyer BJ, Mori TA, Burke V, Mansour J, Patch CS, Tapsell LC, Noakes M, Clifton PA, Barden A, Puddey IB, Beilin LJ, and Howe PR, Impact of foods enriched with n-3 long-chain polyunsaturated fatty acids on erythrocyte n-3 levels and cardiovascular risk factors. *Br J Nutr* 2007;97:749-757.
- [49] Plat J, Jellema A, Ramakers J, and Mensink RP, Weight loss, but not fish oil consumption, improves fasting and postprandial serum lipids, markers of endothelial function, and inflammatory signatures in moderately obese men. *J Nutr* 2007;137:2635-2640.
- [50] Yusof HM, Miles EA, and Calder P, Influence of very long-chain n-3 fatty acids on plasma markers of inflammation in middle-aged men. *Prostaglandins Leukot Essent Fatty Acids* 2008;78:219.
- [51] Vedin I, Cederholm T, Freund Levi Y, Basun H, Garlind A, Faxen Irving G, Jonhagen ME, Vessby B, Wahlund L-O, and Palmblad J, Effects of docosahexaenoic acid-rich n-3 fatty acid supplementation on cytokine release from blood mononuclear leukocytes: the OmegAD study. *Am J Clin Nutr* 2008;87:1616-1622.
- [52] Steele M, Stuchbury G, and Munch G, The molecular basis of the prevention of Alzheimer's disease through healthy nutrition. *Exp Gerontol* 2007;42:28-36.
- [53] Floyd RA, and Hensley K, Oxidative stress in brain aging. Implications for therapeutics of neurodegenerative diseases. *Neurobiol Aging* 2002;23:795-807.

- [54] Migliore L, Fontana I, Trippi F, Colognato R, Coppede F, Tognoni G, Nucciarone B, and Siciliano G, Oxidative DNA damage in peripheral leukocytes of mild cognitive impairment and AD patients. *Neurobiol Aging* 2005;26:567-573.
- [55] Guillot N, Debard C, Calzada C, Vidal H, Lagarde M, and Vericel E, Effects of docosahexaenoic acid on some megakaryocytic cell gene expression of some enzymes controlling prostanoid synthesis. *Biochem Biophys Res Commun* 2008;372:924-928.
- [56] Hashimoto M, Hossain S, Shimada T, Sugioka K, Yamasaki H, Fujii Y, Ishibashi Y, Oka JI, and Shido O, Docosahexaenoic acid provides protection from impairment of learning ability in Alzheimer's disease model rats. *J Neurochem* 2002;81:1084-1091.
- [57] Cole GM, Lim GP, Yang F, Teter B, Begum A, Ma Q, Harris-White ME, and Frautschy SA, Prevention of Alzheimer's disease: Omega-3 fatty acid and phenolic anti-oxidant interventions. *Neurobiol Aging* 2005;26 Suppl 1:133-136.
- [58] Nalsen C, Vessby B, Berglund L, Uusitupa M, Hermansen K, Riccardi G, Rivellese A, Storlien L, Erkkila A, Yla-Herttuala S, Tapsell L, and Basu S, Dietary (n-3) fatty acids reduce plasma F2-isoprostanes but not prostaglandin F2alpha in healthy humans. *J Nutr* 2006;136:1222-1228.
- [59] Allard JP, Kurian R, Aghdassi E, Muggli R, and Royall D, Lipid peroxidation during n-3 fatty acid and vitamin E supplementation in humans. *Lipids* 1997;32:535-541.
- [60] Lagarde M, Docosahexaenoic acid: nutrient and precursor of bioactive lipids. *Eur J Lipid Sci Technol* 2008;110:673-678.
- [61] Bazan NG, Cell survival matters: docosahexaenoic acid signaling, neuroprotection and photoreceptors. *Trends Neurosci* 2006;29:263-271.

- [62] Lukiw WJ, and Bazan NG, Docosahexaenoic acid and the aging brain. *J Nutr* 2008;138:2510-2514.
- [63] Berger J, and Moller DE, The mechanisms of action of PPARs. *Annu Rev Med* 2002;53:409-435.
- [64] Pegorier JP, Le May C, and Girard J, Control of gene expression by fatty acids. *J Nutr* 2004;134:2444S-2449S.
- [65] Harris WS, and Bulchandani D, Why do omega-3 fatty acids lower serum triglycerides? *Curr Opin Lipidol* 2006;17:387-393.
- [66] Zoete V, Grosdidier A, and Michielin O, Peroxisome proliferator-activated receptor structures: ligand specificity, molecular switch and interactions with regulators. *Biochim Biophys Acta* 2007;1771:915-925.
- [67] Heneka MT, and Landreth GE, PPARs in the brain. *Biochim Biophys Acta* 2007;1771:1031-1045.
- [68] Sundararajan S, Jiang Q, Heneka M, and Landreth G, PPARgamma as a therapeutic target in central nervous system diseases. *Neurochem Int* 2006;49:136-144.
- [69] Sampath H, and Ntambi JM, Polyunsaturated fatty acid regulation of gene expression. *Nutr Rev* 2004;62:333-339.
- [70] Sampath H, and Ntambi JM, Polyunsaturated fatty acid regulation of genes of lipid metabolism. *Annu Rev Nutr* 2005;25:317-340.
- [71] Desvergne B, and Wahli W, Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr Rev* 1999;20:649-688.

- [72] Calderon F, and Kim HY, Role of RXR in neurite outgrowth induced by docosahexaenoic acid. *Prostaglandins Leukot Essent Fatty Acids* 2007;77:227-232.
- [73] de Urquiza AM, Liu S, Sjoberg M, Zetterstrom RH, Griffiths W, Sjoval J, and Perlmann T, Docosahexaenoic acid, a ligand for the retinoid X receptor in mouse brain. *Science* 2000;290:2140-2144.
- [74] Lengqvist J, Mata De Urquiza A, Bergman AC, Willson TM, Sjoval J, Perlmann T, and Griffiths WJ, Polyunsaturated fatty acids including docosahexaenoic and arachidonic acid bind to the retinoid X receptor alpha ligand-binding domain. *Mol Cell Proteomics* 2004;3:692-703.
- [75] Etchamendy N, Enderlin V, Marighetto A, Vouimba RM, Pallet V, Jaffard R, and Higuere P, Alleviation of a selective age-related relational memory deficit in mice by pharmacologically induced normalization of brain retinoid signaling. *J Neurosci* 2001;21:6423-6429.
- [76] Feart C, Mingaud F, Enderlin V, Husson M, Alfos S, Higuere P, and Pallet V, Differential effect of retinoic acid and triiodothyronine on the age-related hypo-expression of neurogranin in rat. *Neurobiol Aging* 2005;26:729-738.
- [77] Mingaud F, Mormede C, Etchamendy N, Mons N, Niedergang B, Wietrzyk M, Pallet V, Jaffard R, Krezel W, Higuere P, and Marighetto A, Retinoid hyposignaling contributes to aging-related decline in hippocampal function in short-term/working memory organization and long-term declarative memory encoding in mice. *J Neurosci* 2008;28:279-291.
- [78] Tafti M, and Ghyselinck NB, Functional implication of the vitamin A signaling pathway in the brain. *Arch Neurol* 2007;64:1706-1711.

- [79] Goodman AB, Retinoid receptors, transporters, and metabolizers as therapeutic targets in late onset Alzheimer disease. *J Cell Physiol* 2006;209:598-603.
- [80] Goodman AB, and Pardee AB, Evidence for defective retinoid transport and function in late onset Alzheimer's disease. *Proc Natl Acad Sci U S A* 2003;100:2901-2905.
- [81] Kalmijn S, Launer LJ, Ott A, Witteman JC, Hofman A, and Breteler MM, Dietary fat intake and the risk of incident dementia in the Rotterdam Study. *Ann Neurol* 1997;42:776-782.
- [82] Engelhart MJ, Geerlings MI, Ruitenberg A, Van Swieten JC, Hofman A, Witteman JC, and Breteler MM, Diet and risk of dementia: Does fat matter?: The Rotterdam Study. *Neurology* 2002;59:1915-1921.
- [83] Barberger-Gateau P, Letenneur L, Deschamps V, Peres K, Dartigues JF, and Renaud S, Fish, meat, and risk of dementia: cohort study. *Bmj* 2002;325:932-933.
- [84] Barberger-Gateau P, Raffaitin C, Letenneur L, Berr C, Tzourio C, Dartigues JF, and Alperovitch A, Dietary patterns and risk of dementia: the Three-City cohort study. *Neurology* 2007;69:1921-1930.
- [85] Eskelinen MH, Ngandu T, Helkala EL, Tuomilehto J, Nissinen A, Soininen H, and Kivipelto M, Fat intake at midlife and cognitive impairment later in life: a population-based CAIDE study. *Int J Geriatr Psychiatry* 2008;23:741-747.
- [86] Huang TL, Zandi PP, Tucker KL, Fitzpatrick AL, Kuller LH, Fried LP, Burke GL, and Carlson MC, Benefits of fatty fish on dementia risk are stronger for those without APOE epsilon4. *Neurology* 2005;65:1409-1414.

- [87] Morris MC, Evans DA, Bienias JL, Tangney CC, Bennett DA, Wilson RS, Aggarwal N, and Schneider J, Consumption of fish and n-3 fatty acids and risk of incident Alzheimer disease. *Arch Neurol* 2003;60:940-946.
- [88] Morris MC, Evans DA, Tangney CC, Bienias JL, and Wilson RS, Fish consumption and cognitive decline with age in a large community study. *Arch Neurol* 2005;62:1849-1853.
- [89] Schaefer EJ, Bongard V, Beiser AS, Lamon-Fava S, Robins SJ, Au R, Tucker KL, Kyle DJ, Wilson PW, and Wolf PA, Plasma phosphatidylcholine docosahexaenoic acid content and risk of dementia and Alzheimer disease: the Framingham Heart Study. *Arch Neurol* 2006;63:1545-1550.
- [90] Kalmijn S, van Boxtel MP, Ocke M, Verschuren WM, Kromhout D, and Launer LJ, Dietary intake of fatty acids and fish in relation to cognitive performance at middle age. *Neurology* 2004;62:275-280.
- [91] Barberger-Gateau P, Jutand MA, Letenneur L, Larrieu S, Tavernier B, and Berr C, Correlates of regular fish consumption in French elderly community dwellers: data from the Three-City study. *Eur J Clin Nutr* 2005;59:817-825.
- [92] Nurk E, Drevon CA, Refsum H, Solvoll K, Vollset SE, Nygard O, Nygaard HA, Engedal K, Tell GS, and Smith AD, Cognitive performance among the elderly and dietary fish intake: the Hordaland Health Study. *Am J Clin Nutr* 2007;86:1470-1478.
- [93] Virtanen JK, Siscovick DS, Longstreth WT, Jr., Kuller LH, and Mozaffarian D, Fish consumption and risk of subclinical brain abnormalities on MRI in older adults. *Neurology* 2008;71:439-446.

- [94] Bourre JM, and Paquotte PM, Contributions (in 2005) of marine and fresh water products (finfish and shellfish, seafood, wild and farmed) to the French dietary intakes of vitamins D and B12, selenium, iodine and docosahexaenoic acid: impact on public health. *Int J Food Sci Nutr* 2008;59:491-501.
- [95] Chung H, Nettleton JA, Lemaitre RN, Barr RG, Tsai MY, Tracy RP, and Siscovick DS, Frequency and type of seafood consumed influence plasma (n-3) fatty acid concentrations. *J Nutr* 2008;138:2422-2427.
- [96] Burdge GC, and Calder PC, Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod Nutr Dev* 2005;45:581-597.
- [97] Plourde M, and Cunnane SC, Extremely limited synthesis of long chain polyunsaturates in adults: Implications for their dietary essentiality and use as supplements. *Appl Physiol Nutr Metab* 2007;32:619-634.
- [98] Bégin M, Plourde M, Pifferi F, and Cunnane SC. 2008. What is the link between DHA, progressive cognitive deterioration and Alzheimer's disease in the elderly? *In* *Fat Detection: Taste, Texture, and Post Ingestive Effects*. Montmayeur JP and Le-Coutre J, ed. Taylor and Francis, Boca Raton, FL. in press.
- [99] Willett W. 1998. *Nutritional epidemiology*. Second edition ed. Oxford University Press, New York Oxford.
- [100] Scarmeas N, Stern Y, Tang MX, Mayeux R, and Luchsinger JA, Mediterranean diet and risk for Alzheimer's disease. *Ann Neurol* 2006;59:912-921.
- [101] Arterburn LM, Hall EB, and Oken H, Distribution, interconversion, and dose response of n-3 fatty acids in humans. *Am J Clin Nutr* 2006;83:S1467-1476.

- [102] Sanders TA, Ellis FR, and Dickerson JW, Studies of vegans: the fatty acid composition of plasma choline phosphoglycerides, erythrocytes, adipose tissue, and breast milk, and some indicators of susceptibility to ischemic heart disease in vegans and omnivore controls. *Am J Clin Nutr* 1978;31:805-813.
- [103] Beydoun MA, Kaufman JS, Satia JA, Rosamond W, and Folsom AR, Plasma n-3 fatty acids and the risk of cognitive decline in older adults: the Atherosclerosis Risk in Communities Study. *Am J Clin Nutr* 2007;85:1103-1111.
- [104] Heude B, Ducimetiere P, and Berr C, Cognitive decline and fatty acid composition of erythrocyte membranes--The EVA Study. *Am J Clin Nutr* 2003;77:803-808.
- [105] Laurin D, Verreault R, Lindsay J, Dewailly E, and Holub BJ, Omega-3 fatty acids and risk of cognitive impairment and dementia. *J Alzheimers Dis* 2003;5:315-322.
- [106] Samieri C, Fear C, Letenneur L, Dartigues JF, Peres K, Auriacombe S, Peuchant E, Delcourt C, and Barberger-Gateau P, Low plasma eicosapentaenoic acid and depressive symptomatology are independent predictors of dementia risk. *Am J Clin Nutr* 2008;88:714-721.
- [107] Dullemeijer C, Durga J, Brouwer IA, van de Rest O, Kok FJ, Brummer RJ, van Boxtel MP, and Verhoef P, n 3 fatty acid proportions in plasma and cognitive performance in older adults. *Am J Clin Nutr* 2007;86:1479-1485.
- [108] Corrigan FM, Van Rhijn AG, Ijomah G, McIntyre F, Skinner ER, Horrobin DF, and Ward NI, Tin and fatty acids in dementia. *Prostaglandins Leukot Essent Fatty Acids* 1991;43:229-238.

- [109] Plourde M, Fortier M, Vandal M, Tremblay-Mercier J, Freemantle E, Bégin M, Pifferi F, and Cunnane SC, Unresolved issues in the link between docosahexaenoic acid and Alzheimer disease. *Prostaglandins Leukot Essent Fatty Acids* 2007;77 301-308.
- [110] Soderberg M, Edlund C, Kristensson K, and Dallner G, Fatty acid composition of brain phospholipids in aging and in Alzheimer's disease. *Lipids* 1991;26:421-425.
- [111] Corrigan FM, Horrobin DF, Skinner ER, Besson JA, and Cooper MB, Abnormal content of n-6 and n-3 long-chain unsaturated fatty acids in the phosphoglycerides and cholesterol esters of parahippocampal cortex from Alzheimer's disease patients and its relationship to acetyl CoA content. *Int J Biochem Cell Biol* 1998;30:197-207.
- [112] Han X, Holtzman DM, and McKeel DW, Jr., Plasmalogen deficiency in early Alzheimer's disease subjects and in animal models: molecular characterization using electrospray ionization mass spectrometry. *J Neurochem* 2001;77:1168-1180.
- [113] Guan Z, Wang Y, Cairns NJ, Lantos PL, Dallner G, and Sindelar PJ, Decrease and structural modifications of phosphatidylethanolamine plasmalogen in the brain with Alzheimer disease. *J Neuropathol Exp Neurol* 1999;58:740-747.
- [114] Prasad MR, Lovell MA, Yatin M, Dhillon H, and Markesbery WR, Regional membrane phospholipid alterations in Alzheimer's disease. *Neurochem Res* 1998;23:81-88.
- [115] Skinner ER, Watt C, Besson JA, and Best PV, Differences in the fatty acid composition of the grey and white matter of different regions of the brains of patients with Alzheimer's disease and control subjects. *Brain* 1993;116 (Pt 3):717-725.
- [116] Farooqui AA, Horrocks LA, and Farooqui T, Modulation of inflammation in brain: a matter of fat. *J Neurochem* 2007;101:577-599.

- [117] Brenna JT, and Diau GY, The influence of dietary docosahexaenoic acid and arachidonic acid on central nervous system polyunsaturated fatty acid composition. *Prostaglandins Leukot Essent Fatty Acids* 2007;77:247-250.
- [118] Rapoport SI, Arachidonic acid and the brain. *J Nutr* 2008;138:2515-2520.
- [119] Svennerholm L, Bostrom K, and Jungbjer B, Changes in weight and compositions of major membrane components of human brain during the span of adult human life of Swedes. *Acta Neuropathol (Berl)* 1997;94:345-352.
- [120] van de Rest O, Geleijnse JM, Kok FJ, van Staveren WA, Dullemeijer C, Olderikkert MG, Beekman AT, and de Groot CP, Effect of fish oil on cognitive performance in older subjects: a randomized, controlled trial. *Neurology* 2008;71:430-438.
- [121] Freund-Levi Y, Eriksdotter-Jonhagen M, Cederholm T, Basun H, Faxen-Irving G, Garlind A, Vedin I, Vessby B, Wahlund LO, and Palmblad J, Omega-3 fatty acid treatment in 174 patients with mild to moderate Alzheimer disease: OmegAD study: a randomized double-blind trial. *Arch Neurol* 2006;63:1402-1408.
- [122] Chiu CC, Su KP, Cheng TC, Liu HC, Chang CJ, Dewey ME, Stewart R, and Huang SY, The effects of omega-3 fatty acids monotherapy in Alzheimer's disease and mild cognitive impairment: a preliminary randomized double-blind placebo-controlled study. *Prog Neuropsychopharmacol Biol Psychiatry* 2008;32:1538-1544.
- [123] Johnson EJ, McDonald K, Caldarella SM, Chung HY, Troen AM, and Snodderly DM, Cognitive findings of an exploratory trial of docosahexaenoic acid and lutein supplementation in older women. *Nutr Neurosci* 2008;11:75-83.

- [124] Terano T, Fujishiro S, Ban T, Yamamoto K, Tanaka T, Noguchi Y, Tamura Y, Yazawa K, and Hirayama T, Docosahexaenoic acid supplementation improves the moderately severe dementia from thrombotic cerebrovascular diseases. *Lipids* 1999;34 Suppl:S345-346.
- [125] Boston PF, Bennett A, Horrobin DF, and Bennett CN, Ethyl-EPA in Alzheimer's disease- a pilot study. *Prostaglandins Leukot Essent Fatty Acids* 2004;71:341-346.
- [126] Rogers PJ, Appleton KM, Kessler D, Peters TJ, Gunnell D, Hayward RC, Heatherley SV, Christian LM, McNaughton SA, and Ness AR, No effect of n-3 long-chain polyunsaturated fatty acid (EPA and DHA) supplementation on depressed mood and cognitive function: a randomised controlled trial. *Br J Nutr* 2008;99:421-431.
- [127] van Gelder BM, Tijhuis M, Kalmijn S, and Kromhout D, Fish consumption, n-3 fatty acids, and subsequent 5-y cognitive decline in elderly men: the Zutphen Elderly Study. *Am J Clin Nutr* 2007;85:1142-1147.
- [128] Solfrizzi V, Colacicco AM, D'Introno A, Capurso C, Torres F, Rizzo C, Capurso A, and Panza F, Dietary intake of unsaturated fatty acids and age-related cognitive decline: a 8.5-year follow-up of the Italian Longitudinal Study on Aging. *Neurobiol Aging* 2006;27:1694-1704.
- [129] Rapoport SI, Rao JS, and Igarashi M, Brain metabolism of nutritionally essential polyunsaturated fatty acids depends on both the diet and the liver. *Prostaglandins Leukot Essent Fatty Acids* 2007;77:251-261.
- [130] Mattson MP, Pedersen WA, Duan W, Culmsee C, and Camandola S, Cellular and molecular mechanisms underlying perturbed energy metabolism and neuronal degeneration in Alzheimer's and Parkinson's diseases. *Ann N Y Acad Sci* 1999;893:154-175.

- [131] Freemantle E, Vandal M, Tremblay-Mercier J, Tremblay S, Blachere JC, Begin ME, Brenna JT, Windust A, and Cunnane SC, Omega-3 fatty acids, energy substrates, and brain function during aging. *Prostaglandins Leukot Essent Fatty Acids* 2006;75:213-220.
- [132] Dewailly E, Blanchet C, Gingras S, Lemieux S, and Holub BJ, Fish consumption and blood lipids in three ethnic groups of Quebec (Canada). *Lipids* 2003;38:359-365.
- [133] Kris-Etherton PM, Taylor DS, Yu-Poth S, Huth P, Moriarty K, Fishell V, Hargrove RL, Zhao G, and Etherton TD, Polyunsaturated fatty acids in the food chain in the United States. *Am J Clin Nutr* 2000;71:179S-188S.
- [134] Vandal M, Freemantle E, Tremblay-Mercier J, Plourde M, Fortier M, Bruneau J, Gagnon J, Tremblay S, Bégin M, and Cunnane SC, Plasma omega-3 fatty acid response to a fish oil supplement in the healthy elderly. *Lipids* 2008;43:1085-1089.
- [135] Carver JD, Benford VJ, Han B, and Cantor AB, The relationship between age and the fatty acid composition of cerebral cortex and erythrocytes in human subjects. *Brain Res Bull* 2001;56:79-85.
- [136] Conklin SM, Gianaros PJ, Brown SM, Yao JK, Hariri AR, Manuck SB, and Muldoon MF, Long-chain omega-3 fatty acid intake is associated positively with corticolimbic gray matter volume in healthy adults. *Neurosci Lett* 2007;421:209-212.
- [137] Itomura M, Fujioka S, Hamazaki K, Kobayashi K, Nagasawa T, Sawazaki S, Kirihaara Y, and Hamazaki T, Factors influencing EPA+DHA levels in red blood cells in Japan. *In Vivo* 2008;22:131-135.
- [138] Jofre-Monseny L, Minihane AM, and Rimbach G, Impact of apoE genotype on oxidative stress, inflammation and disease risk. *Mol Nutr Food Res* 2008;52:131-145.

- [139] Greenwood PM, Lambert C, Sunderland T, and Parasuraman R, Effects of apolipoprotein E genotype on spatial attention, working memory, and their interaction in healthy, middle-aged adults: results From the National Institute of Mental Health's BIOCARD study. *Neuropsychology* 2005;19:199-211.
- [140] Whalley LJ, Deary IJ, Starr JM, Wahle KW, Rance KA, Bourne VJ, and Fox HC, n-3 Fatty acid erythrocyte membrane content, APOE varepsilon4, and cognitive variation: an observational follow-up study in late adulthood. *Am J Clin Nutr* 2008;87:449-454.
- [141] Anil E, The impact of EPA and DHA on blood lipids and lipoprotein metabolism: influence of apoE genotype. *Proc Nutr Soc* 2007;66:60-68.
- [142] Minihane AM, Khan S, Leigh-Firbank EC, Talmud P, Wright JW, Murphy MC, Griffin BA, and Williams CM, ApoE polymorphism and fish oil supplementation in subjects with an atherogenic lipoprotein phenotype. *Arterioscler Thromb Vasc Biol* 2000;20:1990-1997.
- [143] Ordovas JM, Gene-diet interaction and plasma lipid responses to dietary intervention. *Biochem Soc Trans* 2002;30:68-73.
- [144] Plourde M, Vohl MC, Caron-Dorval D, Couture P, Lemieux S, and Cunnane SC, Apolipoprotein E and PPAR- α L162V polymorphisms influence plasma omega-3 incorporation International Society for the Study of Fatty Acids and Lipids 2008; conference abstract.
- [145] Irving GF, Freund-Levi Y, Eriksdotter-Jonhagen M, Basun H, Brismar K, Hjorth E, Palmblad J, Vessby B, Vedin I, Wahlund LO, and Cederholm T, Omega-3 fatty acid supplementation effects on weight and appetite in patients with Alzheimer's disease: the omega-3 Alzheimer's disease study. *J Am Geriatr Soc* 2009;57:11-17.

- [146] Tremblay F, Lavigne C, Jacques H, and Marette A, Role of dietary proteins and amino acids in the pathogenesis of insulin resistance. *Annu Rev Nutr* 2007;27:293-310.
- [147] Ouellet V, Weisnagel SJ, Marois J, Bergeron J, Julien P, Gougeon R, Tchernof A, Holub BJ, and Jacques H, Dietary cod protein reduces plasma C-reactive protein in insulin-resistant men and women. *J Nutr* 2008;138:2386-2391.
- [148] Xu W, Qiu C, Gatz M, Pedersen NL, Johansson B, and Fratiglioni L, Mid- and late-life diabetes in relation to the risk of dementia: a population-based twin study. *Diabetes* 2009;58:71-77.
- [149] Pasinetti GM, and Eberstein JA, Metabolic syndrome and the role of dietary lifestyles in Alzheimer's disease. *J Neurochem* 2008;106:1503-1514.
- [150] Souci S, Fachman W, and Kraut H. 2000. Food composition and nutrition tables medpharm Scientific publishers, Stuttgart.
- [151] Berr C, Akbaraly T, Arnaud J, Hininger I, Roussel AM, and Barberger Gateau P, Increased selenium intake in elderly high fish consumers may account for health benefits previously ascribed to omega-3 Fatty acids. *J Nutr Health Aging* 2009;13:14-18.
- [152] Norman AW, A vitamin D nutritional cornucopia: new insights concerning the serum 25-hydroxyvitamin D status of the US population. *Am J Clin Nutr* 2008;88:1455-1456.
- [153] Husson M, Enderlin V, Delacourte A, Ghenimi N, Alfos S, Pallet V, and Higuieret P, Retinoic acid normalizes nuclear receptor mediated hypo-expression of proteins involved in beta-amyloid deposits in the cerebral cortex of vitamin A deprived rats. *Neurobiol Dis* 2006;23:1-10.

- [154] Clarke R, B-vitamins and prevention of dementia. *Proceedings of the Nutrition Society* 2008;67:75-81.
- [155] Alessandri JM, Guesnet P, Vancassel S, Astorg P, Denis I, Langelier B, Aid S, Poumes-Ballihaut C, Champeil-Potokar G, and Lavalie M, Polyunsaturated fatty acids in the central nervous system: evolution of concepts and nutritional implications throughout life. *Reprod Nutr Dev* 2004;44:509-538.
- [156] Deschamps V, Barberger-Gateau P, Peuchant E, and Orgogozo JM, Nutritional factors in cerebral aging and dementia: epidemiological arguments for a role of oxidative stress. *Neuroepidemiology* 2001;20:7-15.
- [157] Morris MC, Evans DA, Tangney CC, Bienias JL, and Wilson RS, Associations of vegetable and fruit consumption with age-related cognitive change. *Neurology* 2006;67:1370-1376.
- [158] Letenneur L, Proust-Lima C, Le Gouge A, Dartigues J, and Barberger-Gateau P, Flavonoid Intake and Cognitive Decline over a 10-Year Period. *Am J Epidemiol* 2007;165:1364-1371.
- [159] Arendash GW, Jensen MT, Salem Jr N, Hussein N, Cracchiolo J, Dickson A, Leighty R, and Potter H, A diet high in omega-3 fatty acids does not improve or protect cognitive performance in Alzheimer's transgenic mice. *Neuroscience* 2007;149:286.
- [160] Liou YA, King DJ, Zibrik D, and Innis SM, Decreasing Linoleic Acid with Constant α -Linolenic Acid in Dietary Fats Increases (n-3) Eicosapentaenoic Acid in Plasma Phospholipids in Healthy Men. *J Nutr* 2007;137:945-952.

[161] Calon F, Lim GP, Morihara T, Yang F, Ubeda O, Salem N, Jr., Frautschy SA, and Cole GM, Dietary n-3 polyunsaturated fatty acid depletion activates caspases and decreases NMDA receptors in the brain of a transgenic mouse model of Alzheimer's disease. *Eur J Neurosci* 2005;22:617-626.

[162] Calon F, Lim GP, Yang F, Morihara T, Teter B, Ubeda O, Rostaing P, Triller A, Salem N, Jr., Ashe KH, Frautschy SA, and Cole GM, Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model. *Neuron* 2004;43:633-645.

[163] Lim GP, Calon F, Morihara T, Yang F, Teter B, Ubeda O, Salem N, Jr., Frautschy SA, and Cole GM, A diet enriched with the omega-3 fatty acid docosahexaenoic acid reduces amyloid burden in an aged Alzheimer mouse model. *J Neurosci* 2005;25:3032-3040.

[164] Green KN, Martinez-Coria H, Khashwji H, Hall EB, Yurko-Mauro KA, Ellis L, and LaFerla FM, Dietary docosahexaenoic acid and docosapentaenoic acid ameliorate amyloid- β and tau pathology via a mechanism involving presenilin 1 levels. *J Neurosci* 2007;27:4385-4395.

[165] Hashimoto M, Hossain S, Agdul H, and Shido O, Docosahexaenoic acid-induced amelioration on impairment of memory learning in amyloid beta-infused rats relates to the decreases of amyloid beta and cholesterol levels in detergent-insoluble membrane fractions. *Biochim Biophys Acta* 2005;1738:91-98.

[166] Hashimoto M, Hossain S, Shimada T, and Shido O, Docosahexaenoic acid-induced protective effect against impaired learning in amyloid beta-infused rats is associated with increased synaptosomal membrane fluidity. *Clin Exp Pharmacol Physiol* 2006;33:934-939.

- [167] Bons N, Rieger F, Prudhomme D, Fisher A, and Krause KH, Microcebus murinus: a useful primate model for human cerebral aging and Alzheimer's disease? *Genes Brain Behav* 2006;5:120-130.
- [168] Kalpouzos G, Chetelat G, Baron JC, Landeau B, Mevel K, Godeau C, Barre L, Constans JM, Viader F, Eustache F, and Desgranges B, Voxel-based mapping of brain gray matter volume and glucose metabolism profiles in normal aging. *Neurobiol Aging* 2009;30:112-124.
- [169] Kalpouzos G, Eustache F, de la Sayette V, Viader F, Chetelat G, and Desgranges B, Working memory and FDG-PET dissociate early and late onset Alzheimer disease patients. *J Neurol* 2005;252:548-558.
- [170] Bourre JM, Francois M, Youyou A, Dumont O, Piciotti M, Pascal G, and Durand G, The effects of dietary alpha-linolenic acid on the composition of nerve membranes, enzymatic activity, amplitude of electrophysiological parameters, resistance to poisons and performance of learning tasks in rats. *J Nutr* 1989;119:1880-1892.
- [171] Ximenes da Silva A, Lavalie F, Gendrot G, Guesnet P, Alessandri JM, and Lavalie M, Glucose transport and utilization are altered in the brain of rats deficient in n-3 polyunsaturated fatty acids. *J Neurochem* 2002;81:1328-1337.
- [172] Pifferi F, Roux F, Langelier B, Alessandri JM, Vancassel S, Jouin M, Lavalie M, and Guesnet P, (n-3) polyunsaturated fatty acid deficiency reduces the expression of both isoforms of the brain glucose transporter GLUT1 in rats. *J Nutr* 2005;135:2241-2246.

- [173] Pifferi F, Jouin M, Alessandri JM, Haedke U, Roux F, Perriere N, Denis I, Lavalie M, and Guesnet P, n-3 Fatty acids modulate brain glucose transport in endothelial cells of the blood-brain barrier. *Prostaglandins Leukot Essent Fatty Acids* 2007;77:279-286.
- [174] Reiman EM, Chen K, Alexander GE, Caselli RJ, Bandy D, Osborne D, Saunders AM, and Hardy J, Functional brain abnormalities in young adults at genetic risk for late-onset Alzheimer's dementia. *Proc Natl Acad Sci U S A* 2004;101:284-289.
- [175] Maclean CH, Issa AM, Newberry SJ, Mojica WA, Morton SC, Garland RH, Hilton LG, Traina SB, and Shekelle PG, Effects of omega-3 fatty acids on cognitive function with aging, dementia, and neurological diseases. *Evid Rep Technol Assess (Summ)* 2005:1-3.
- [176] Kalmijn S, Feskens EJ, Launer LJ, and Kromhout D, Polyunsaturated fatty acids, antioxidants, and cognitive function in very old men. *Am J Epidemiol* 1997;145:33-41.
- [177] Beydoun MA, Kaufman JS, Sloane PD, Heiss G, and Ibrahim J, n-3 Fatty acids, hypertension and risk of cognitive decline among older adults in the Atherosclerosis Risk in Communities (ARIC) study. *Public Health Nutr* 2008;11:17-29.
- [178] Tilvis RS, Erkinjuntti T, Sulkava R, and Miettinen TA, Fatty acids of plasma lipids, red cells and platelets in Alzheimer's disease and vascular dementia. *Atherosclerosis* 1987;65:237-245.
- [179] Conquer JA, Tierney MC, Zecevic J, Bettger WJ, and Fisher RH, Fatty acid analysis of blood plasma of patients with Alzheimer's disease, other types of dementia, and cognitive impairment. *Lipids* 2000;35:1305-1312.

- [180] Manzato E, Roselli della Rovere G, Zambon S, Romanato G, Corti MC, Sartori L, Baggio G, and Crepaldi G, Cognitive functions are not affected by dietary fatty acids in elderly subjects in the Pro.V.A. study population. *Aging Clin Exp Res* 2003;15:83-86.
- [181] Tully AM, Roche HM, Doyle R, Fallon C, Bruce I, Lawlor B, Coakley D, and Gibney MJ, Low serum cholesteryl ester-docosahexaenoic acid levels in Alzheimer's disease: a case-control study. *Br J Nutr* 2003;89:483-489.
- [182] Cherubini A, Andres-Lacueva C, Martin A, Lauretani F, Iorio AD, Bartali B, Corsi A, Bandinelli S, Mattson MP, and Ferrucci L, Low plasma N-3 fatty acids and dementia in older persons: the InCHIANTI study. *J Gerontol A Biol Sci Med Sci* 2007;62:1120-1126.
- [183] Wang W, Shinto L, Connor WE, and Quinn JF, Nutritional biomarkers in Alzheimer's disease: the association between carotenoids, n-3 fatty acids, and dementia severity. *J Alzheimers Dis* 2008;13:31-38.
- [184] Brooksbank BW, and Martinez M, Lipid abnormalities in the brain in adult Down's syndrome and Alzheimer's disease. *Mol Chem Neuropathol* 1989;11:157-185.
- [185] Suzuki H, Morikawa Y, and Takahashi H, Effect of DHA oil supplementation on intelligence and visual acuity in the elderly. *World Rev Nutr Diet* 2001;88:68-71.
- [186] Kotani S, Sakaguchi E, Warashina S, Matsukawa N, Ishikura Y, Kiso Y, Sakakibara M, Yoshimoto T, Guo J, and Yamashima T, Dietary supplementation of arachidonic and docosahexaenoic acids improves cognitive dysfunction. *Neurosci Res* 2006;56:159-164.
- [187] Terry PD, Rohan TE, and Wolk A, Intakes of fish and marine fatty acids and the risks of cancers of the breast and prostate and of other hormone-related cancers: a review of the epidemiologic evidence. *Am J Clin Nutr* 2003;77:532-543.

Table 1

Prospective observational studies relating fish or dietary DHA consumption to risk of dementia or cognitive decline.

Study	N	Age (years)	Food or nutrient	Outcome	Results (multivariate models)
Rotterdam Study (The Netherlands) [81]	5386	≥ 55	Fish Total fat SFA	All cause dementia AD VaD Mean follow-up 2.1 years	Protective association of fish consumption (at least 18.5 g/d) with all-cause dementia and AD Increased risk of vascular dementia with total fat and saturated fat
Rotterdam Study (The Netherlands) [82]	5395	≥ 55	Total, SFA, trans fat, MUFA, total PUFA, total ω6 PUFA, total ω3 PUFA	All cause dementia AD VaD Mean follow-up 6.0 years	No association with any class of fatty acids
PAQUID (France) [83]	1416	≥ 68	Fish	All cause dementia AD 7 years of follow-up	Protective association of at least weekly fish consumption against all-cause dementia and AD
Chicago Health and Aging Project (USA) [87]	815	65 - 94	Fish Total ω3 PUFA EPA, DHA	AD Mean follow-up 3.9 years	Protective association with fish consumption, total ω3 PUFA and DHA No association with EPA
Chicago Health and Aging Project (USA) [88]	3718	≥ 65	Fish Total ω3 PUFA EPA, DHA	Cognitive decline over 6 years (global measure from 4 standardized tests)	At least weekly fish consumption associated with slower cognitive decline No association with ω3 PUFA, EPA or DHA
Cardiovascular Health Cognition Study [86]	2233	≥ 65	Fish	All cause dementia AD VaD	Protective association with nonfried fish for AD only in ApoE4 negative (N=1570); NS when additionally adjusted for education and income No association in ApoE4 positive (N=474)

Three City Study (France) [84]	8085	≥ 65	Fish	All cause dementia AD	Fish associated with lower risk of all-cause dementia only in ApoE4 negative (N=5944) No association in ApoE4 positive (N=1479) No significant association with AD
Zutphen Elderly Study [176]	342	69-89	Fish	Cognitive decline over 3 years in MMSE score	Fish inversely but not significantly associated with cognitive decline
Zutphen Elderly Study [127]	210	70 - 89	Fish EPA+DHA	Cognitive decline over 5 years	Fish and EPA+DHA intake associated with less cognitive decline
Cardiovascular Risk Factors, Aging and Dementia Study (Finland) [85]	1449	60 - 80	Fish SFA PUFA	Cognitive performance 21 years later MCI	SFA associated with poorer cognitive function and increased risk of MCI Higher intake of PUFA and fish associated with better performances
Framingham Heart Study [89]	488	76	Fish DHA	All-cause dementia AD Mean follow-up 9.1 years	No significant association
Atherosclerosis Risk in Communities Study [177]	7814	50-65	Total ω3 and ω6 PUFA Long-chain ω3 and ω6 PUFA	Cognitive decline over 9 years	Higher long-chain ω3 PUFA and balanced ω6/ω3 ratio associated with lower risk of cognitive decline, especially for verbal fluency and among hypertensives

PAQUID: Personnes Agées QUID

SFA: Saturated fat

MUFA: Mono-unsaturated fat

PUFA: Polyunsaturated fat

AD: Alzheimer disease

MCI: Mild Cognitive Impairment

Table 2

Blood docosahexaenoic acid (% of total fatty acids) in relation to cognitive status in the elderly.

Reference	Type of study, duration follow-up	Cognitive status	N	Age (y) Mean	DHA
A. PLASMA PL					
Tilvis et al., [178]	Cross-sectional	AD	11	69	7.4 ± 0.6
		VD	19	73	6.4 ± 0.4
Corrigan et al. [108]	Cross-sectional	Control	49	74	5.6 ± 0.6
		MID	6	81	5.5 ± 0.2
		AD	36	81	4.4 ± 0.9
Conquer et al. [179]	Cross-sectional	Control	19	77	4.6 ± 0.4
		CIND	27	83	3.7 ± 0.2
		AD	13	83	3.1 ± 0.2
Laurin et al.[105]	Cross-sectional	Control - all	79	77	2.1 ± 0.8
		ApoE4 non-carriers	65	N/A	2.1 ± 0.6
		ApoE4 carriers	13	N/A	N/A
		CIND	43	79	2.1 ± 0.8
		Demented - all	52	81	2.3 ± 0.8
		ApoE4 non-carriers	28	N/A	2.4 ± 0.7
		ApoE4 carriers	24	N/A	N/A
Laurin et al. [105]	Prospective, 5 y	Control	52	-	2.0 ± 0.7
		CIND	16	-	2.1 ± 0.8
		Demented	11	-	2.7 ± 1.2
Manzato et al., [180]	Cross-sectional	Control	98	72	3.3 ± 1.1
		CIND	93	82	3.1 ± 1.1
Beydoun et al. [103]	Prospective, 6 y	Control	2111	56	2.9 ± 0.9
		CD	140	57	3.0 ± 0.9
B. PLASMA PC					
Conquer et al. [179]	Cross-sectional	Control	19	77	4.9 ± 0.4

		CIND	27	83	3.6 ± 0.2
		AD	13	83	3.2 ± 0.2
Schaefer et al. [89]	Prospective, 9 y	CD	899	76	>4.2% = lower risk for CD

C. PLASMA CE

Tilvis et al., [178]	Cross-sectional	AD	11	69	1.3 ± 0.1
		VD	19	73	1.1 ± 0.1
Corrigan et al. [108]	Cross-sectional	Control	49	74	0.4 ± 0.4
		MID	6	81	2.0 ± 1.0
		AD	36	81	0.6 ± 0.5
Tully et al. [181]	Cross-sectional	Control	45	69	1.2 ± 0.9
		CD ¹	42	76	0.6 ± 0.5
		AD ²	42	75	0.7 ± 0.7
Beydoun et al. [103]	Prospective, 6 y	Control	2111	56	0.5 ± 0.2
		CD	140	57	0.5 ± 0.2
Dullemeijer et al. [107]	Prospective, 3 y		404	60	every doubling in ω 3 PUFA proportion ↓ rate (9-12%) in speed-related cognitive domains
Dullemeijer et al. [107]	Cross-sectional		807	60	No association with any of the 5 cognitive domains

D. PLASMA TOTAL FA

Cherubini et al., [182]	Cross-sectional	Control	725	74	2.3 (2.2 - 2.3)
		CIND	153	81	2.2 (2.1 - 2.3)
		Dementia	57	85	2.0 (1.8 - 2.2)
Samieri et al. [106]	Prospective, 4 y	Control	1149	74	2.4 ± 0.8
		Dementia	65	78	2.2 ± 0.8

E. ERYTHROCYTES

Tilvis et al., [178]	Cross-sectional	AD	11	69	10.6 ± 1.3
		VD	19	73	5.6 ± 0.8
Corrigan et al., [108]	Cross-sectional	Control	49	74	3.2 ± 2.5
		MID	6	81	5.6 ± 1.8
		AD	36	81	4.4 ± 2.2
Heude et al. [104]	Prospective, 4 y	Control	219	69	6.3 ± 1.1
		CD	27	69	5.9 ± 1.0
Boston et al. [125]	Cross-sectional	Control	10	-	41.2 ± 20.9 ³
		AD	22	81	35.3 ± 21.5 ³
Wang et al. [183]	Cross-sectional	CD	13	77	5.4 ± 1.6
		AD	10	75	4.2 ± 1.2

AD: Alzheimer's disease; ApoE4: Apolipoprotein E ε4; CE: cholesteryl esters; CIND: cognitive impaired non demented; CD: cognitive decline; MID: multi-infarct dementia; PC: phosphatidylcholine; PUFA: polyunsaturated fatty acids; TPL: total phospholipids; VD: Vascular dementia

¹ 1st quartile of AD

² 4th quartile of AD

³ µg/g

Table 3

Docosahexaenoic acid in brain lipids in the healthy elderly compared to Alzheimer's disease (AD).

Reference	Cognitive status	N	Age (y)	Docosahexaenoic Acid ^a			
				PE	PC	Total PL	Free
FRONTAL CORTEX							
Brooksbank et al., [184]	Control	6	73	25,9	2,5		
	AD	6	75	25,9	2,5		
Soderberg et al. [110] ¹	Control	8-10	n/a	23.5	1.4		
	AD	8-10	n/a	12.6	0.8		
Soderberg et al. [110] ²	Control	8-10	n/a	4.6	TR		
	AD	8-10	n/a	2.0	TR		
Guan et al. [113]	Control	13	72	2129 ^b	491 ^b		
	AD	15	80	2512	361		
Skinner et al. [115] ¹	Control	10	68				18.6
	AD	9	79				17.3
Skinner et al. [115] ²	Control	8	68				3.6
	AD	7	79				4.6
PARAHIPPOCAMPUS							
Corrigan et al. [111]	Control	6	73	20.7	1.6		
	AD	8	77	20.5	1.4		
Skinner et al. [115] ¹	Control	10	68				16.9
	AD	12	79				16.6
Skinner et al. [115] ²	Control	8	68				5.1
	AD	7	79				5.0
HIPPOCAMPUS							
Soderberg et al. [110]	Control	8-10	n/a	16.9	0.9		
	AD	8-10	n/a	7.9	0.6		
Prasad et al. [114]	Control	9	78	1017 ^b	121 ^b		
	AD	9	78	557	84		
Lukiw et al., [19]	Control	6	69				55 ^c
	AD	6	70				25 ^c

AD: Alzheimer's disease; n/a: not available; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PL: phospholipids; TR: Trace

^a Unless otherwise noted, docosahexaenoic acid values are % of total fatty acids

^b nmol/g wet tissue

^c estimated from Figure 5 of Lukiw et al., [19], nmol/mg protein

¹ Gray matter

² White matter

Table 4

Impact of ω 3 PUFA supplementation in the elderly with dementia or declining cognitive function.

Reference	Cognitive status	N	Age (y)	Supplement	Dose (g)	Duration (mo)	Measures
Terano et al. [124]	DTCD	10	83	None		12	6% lower scores
	DTCD	10	83	DHA	4.3	12	17% higher scores
Suzuki et al. [185]	Control	8	78	DHA + EPA	0.6 + 0.5	6	75% of subjects improved ^a
	CD	22	78	DHA + EPA	0.6 + 0.5	6	55% of subjects improved ^a
Kotani et al. [186]	MCI	9	70	P (olive oil)	0.24	3	No change
	MCI	12	67	DHA + ARA _d	0.24	3	8 - 15% higher scores ^b
	AD	8	67	DHA + ARA _d	0.24	3	No change
Freund-Levi et al. [121]	AD	85	73	P (corn oil)/DHA + EPA	4.0/ 1.7 + 0.6	6/6	No change ^c
	AD	89	73	DHA + EPA	1.7 + 0.6	12	No change ^c
Chiu et al., [122]				DHA + EPA	0.7 + 1.1	6	
	CIND	14	70-81				No change
	CIND	9	70-81	P (olive oil)	1.8	6	significant improvement in ADAS-cog test compared to P
	AD	10	70-81	DHA + EPA	0.7 + 1.1	6	No change
	AD	13	70-81	P (olive oil)	1.8	6	No change
Boston et al [125]	AD	19	81	Ethyl-EPA	1	3	No change

AD: Alzheimer's disease; ARA: arachidonic acid; CD: cognitive decline; DHA: docosahexaenoic acid; DTCD dementia from thrombotic cerebrovascular disease EPA: eicosapentaenoic acid; MCI: mildly cognitive impaired; MMD: mild to moderate dementia; NS: not significant; P: placebo.

^a % of subjects with improved cognitive score

^b attention and memory tests

^c Except in subjects with scores >27 on the Mini-mental state exam

^d In unknown proportions

FIGURE LEGENDS

Figure 1

Blood docosahexaenoic acid (DHA; % control) as calculated from the values reported in the published prospective studies listed in Table 2. As a % of each study's own controls, overall mean plasma DHA value for the two data sets showing all causes of dementia including AD was 114 ± 22 (mean \pm SD; black bars). For the four data sets reporting other forms of cognitive decline excluding dementia or AD, it was 100 ± 5 (gray bars). As reported in the original studies, only the data of Heude et al [104] reported DHA significantly different from each study's own control values (100%; * $p < 0.05$). Note that two other published prospective studies were excluded from this analysis since they had no control group *per se* [89, 107]. CE: plasma cholesteryl esters, PL: plasma phospholipids, RBC: red blood cell total lipids, TFA: plasma total fatty acids.

Figure 2

Blood docosahexaenoic acid (DHA; % control) in all causes of dementia or dementia excluding Alzheimer's disease (AD) as calculated from the values reported in the published prospective studies listed in Table 2. DHA values different from each study's own control values (100%) as reported in the original study are identified by an asterisk (* $p < 0.05$). As a % of control, overall mean blood DHA value for the nine data sets reporting all cause dementia including AD was 93 ± 31 (mean \pm SD; black bars). For the nine data sets reporting other forms of cognitive decline except AD, it was 141 ± 131 (gray bars). If the data of Corrigan et al (1991) [108] are considered to be an «outlier», the overall means for all causes of dementia including AD become 79 ± 20 and 82 ± 20 for other forms of cognitive decline. Hence, even within studies, these cross-sectional studies on various forms of cognitive decline show wide

variability in blood DHA relative to controls. Note that two other published cross-sectional studies were excluded from this analysis since they had no control group *per se* [183, 187]. CE: plasma cholesteryl esters, PC: plasma phosphatidylcholine, PL: plasma phospholipids, RBC: Red blood cell total lipids, TFA: plasma total fatty acids.

Figure 3.

Docosahexaenoic acid (DHA) in phosphatidylethanolamine (PE), phosphatidylcholine (PC), total phospholipids (TPL), or as free DHA from Alzheimer's disease brain samples. In two studies, gray matter (A) was distinguished from white matter (B). Data are shown as % control as calculated from the original data reported in Table 3 (* $p < 0.05$, as reported in the original study). Compared to controls (100%), lower DHA was most consistently seen in hippocampus (3 studies), was not significantly different from control in parahippocampus (2 studies) or frontal cortex (4 studies), which had the most variability.





